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Dehydration Tolerance of Several Populus deltoides Clones

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DEHYDRATION TOLERANCE OF SEVERAL *POPULUS DELTOIDES* CLONES

by

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DEHYDRATION TOLERANCE OF SEVERAL *POPULUS DELTOIDES* CLONES

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Drought resistance of five field-grown eastern cottonwood (*Populus deltoides* Bartr.) clones was examined during two growing seasons. In the first experiment, clonal and seasonal differences in predawn leaf water potential (Ψ_w), predawn leaf osmotic potential (Ψ_s), dry weight fraction (DWF), and height growth were investigated. The second experiment examined clonal and seasonal differences in electrolyte leakage.

During dry periods, DWF values increased and Ψ_s values declined to as low as -2.1 MPa from a high of -1.4 MPa. There were significant negative correlation coefficients between DWF and Ψ_s for most clones. High Ψ_w values were found for clones from Nebraska ("Platte") and Missouri ("Mighty Mo") in 1988. Platte showed significantly higher Ψ_s values than "Tippecanoe" (Indiana clone) for most dates in 1988. There were no differences between Platte and Tippecanoe in Ψ_s for most dates in 1989 and both had lower values than "Ohio Red" (Ohio clone).

Ohio Red showed the lowest height growth in 1988. No significant height differences were found among clones after June 1989.

Injury index (I_d) was calculated from conductivity changes of a solution due to electrolyte leakage from leaf tissue during rehydration. Values for I_d declined when samples were measured after dry periods for all clones except Platte. Platte

showed consistently low I_d values in 1988. In 1989, when I_d values increased following favorable weather conditions, Platte and Tippecanoe had significantly lower I_d than Ohio Red. There were no significant differences in I_d among clones when plants were sampled after a dry period, indicating that all clones had drought-hardened. On dates that both Ψ_s and I_d were measured in 1989, Platte and Tippecanoe had significantly lower I_d and Ψ_s than Ohio Red.

It is concluded that all clones studied have drought tolerance characteristics such as lower Ψ_s, higher DWF, and lower leakage during dry periods. Platte and Tippecanoe were more dehydration tolerant than Ohio Red. There were no significant differences between Mighty Mo and Ohio Red in drought tolerance.

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INTRODUCTION

Eastern cottonwood (*Populus deltoides* Bartr.) is one of the most valuable species of the *Aigeros* section of the poplars (Herpka 1976). Although only 1.5% of Nebraska's total land area is forested, the cottonwood type contains the second largest timber volume and comprises 15% of Nebraska's commercial forest area. Eastern cottonwood occurs mainly on mesic and hydromesic sites in Nebraska (Raile 1986). It is however, often planted on more xeric sites for windbreaks and other plantings. Its sprouting characteristics together with its fast growth also make it a possible fuelwood species (Dutrow 1976).

Water deficit is one of the major factors limiting the growth of land plants (Levitt 1980, Kramer 1983). Water is often limiting in Nebraska due to lack of precipitation and high evaporative demand (Wilhite 1981, Rosenberg *et al.* 1983). Considering the high moisture demand of *P. deltoides* (Regehr *et al.* 1975), it is important to identify drought resistant characteristics for selection of genotypes.

Studies have shown that there is variation in drought resistance among provenances of eastern cottonwood (Kelliher and Tauer 1980, Coleman 1982). Kelliher and Tauer (1980) found differences in stomatal resistance between clones from dry and wet sites. Clonaru and Saeed (1976) reported differences in establishment and growth among different provenances planted in Iraq, with a semi-arid to arid climate.

Higher plants have two mechanisms of drought tolerance: dehydration avoidance and dehydration tolerance (Kramer 1980, Jones *et al.* 1981, Clarke and Durley 1981). Although there is some literature on water relations of poplars in

general and of eastern cottonwood in particular, no study has been done on dehydration tolerance of the species. In fact, such studies have been limited to lower plants such as mosses and a few annual crop plants. However, dehydration tolerance mechanisms are known to occur in higher plants (Levitt 1980) and were recently shown in woody plants. Martin *et al.* (1987) used electrolyte leakage measurements to calculate an injury index and rank six woody species according to their dehydration tolerance. This method has also been used in cold, heat, and drought tolerance studies with several species. Sullivan and Ross (1979) used this method to compare heat and drought tolerance of two sorghum (*Sorghum bicolor* L.) varieties.

The purpose of this study was to investigate 1) the seasonal and clonal differences in water relations, and 2) the existence and extent of dehydration tolerance in several *P. deltoides* clones using the electrolyte leakage method.

LITERATURE REVIEW

I. DROUGHT RESISTANCE TERMINOLOGY

Drought stress is defined by Levitt (1980) as water stress due to lack of rain. He notes that both water stress and drought stress are used interchangeably, though he prefers the latter because it refers only to a deficit and not to an excess of water.

Drought resistance is a general term used to describe the mechanisms by which plants survive periods of dry weather (Kramer 1980, Jones *et al.* 1981). As

noted by Kramer (1983), there are differences among authors in the classification of drought resistance mechanisms. Kramer (1983) classified drought resistance mechanisms in two groups: a) drought avoidance, when plants are not subjected to meteorological drought, i.e. plants complete their life cycle before the dry season; and b) drought tolerance, when plants are able to postpone and/or tolerate dehydration. Dehydration postponement mechanisms either reduce water loss or increase absorption by leaf or root modifications, respectively. Osmotic adjustment and protoplasmic tolerance are two dehydration tolerance mechanisms described by Kramer (1983).

Levitt (1980, 1985, 1986) described drought escape and drought avoidance equivalent to Kramer's (1983) drought avoidance and dehydration postponement, respectively. Kramer (1980, p. 14) criticized Levitt's (1980) usage of drought avoidance because "... such plants do not avoid drought; rather, they possess various adaptations that enable them to tolerate it." According to Levitt (1980), drought escape should not be considered a drought resistance mechanism because the plants do not experience water deficit.

Jones *et al.* (1981) also classified drought resistance mechanisms into three primary types. These are defined as:

1) Drought escape-- the ability of a plant to complete its life cycle before a serious water deficit develops, e.g. ephemerals. Kramer (1983) used this group of plants as an example for drought avoidance.

2) Drought tolerance at high tissue water potential-- the ability of a plant to endure periods of water deficit while maintaining a high tissue water potential.

Among the mechanisms are maintenance of water uptake by root growth and/or reduction of water loss by stomatal control. This is similar to Kramer's dehydration postponement and Levitt's (1980) drought avoidance. Jones *et al.* (1981) commented that plants with these mechanisms do not avoid drought, but avoid tissue dehydration.

3) Drought tolerance at low tissue water potential-- the ability of a plant to endure water deficits at low tissue water potential. The mechanisms are maintenance of turgor and/or desiccation tolerance by means of protoplasmic resistance, osmotic adjustment, or increase in cell wall elasticity. These are recognized as mechanisms of dehydration tolerance by Kramer (1983). Levitt (1980) subdivided this group further into dehydration avoidance (turgor maintenance) and dehydration tolerance (protoplasmic tolerance).

It is evident from this discussion that response mechanisms of dehydration tolerance and dehydration avoidance differ depending on whose classification system is used. Levitt (1980, 1985, 1986) consistently considered osmotic adjustment as a dehydration avoidance mechanism while according to Kramer (1983) it is a dehydration tolerance mechanism. Pallardy (1981) called osmotic adjustment a desiccation tolerance response. Steponkus *et al.* (1980) reported that osmotic adjustment could be viewed as a mechanism for both types. It can be considered a tolerance mechanism from a decreased soil water perspective and an avoidance or postponement mechanism from a cellular turgor perspective.

In this paper, Levitt's (1980) definition of drought tolerance and drought avoidance are used.

II. IMPORTANCE AND INTERRELATIONS OF DROUGHT RESISTANCE MECHANISMS

Dehydration tolerance is more commonly reported in lower plants than in higher plants (Bewley 1979, Clarke and Durley 1981), probably due to limited research with the latter. In agricultural crops where productivity and yield are important, dehydration tolerance is considered of little significance (Sullivan and Eastin 1974, Jones *et al.* 1981) because when crops are severely stressed they usually have little economic value (Kramer 1980). However, dehydration tolerance capacity is of importance in selection for breeding (Sullivan and Eastin 1974, Sullivan and Ross 1979, Krishnamani *et al.* 1984) as one of a complex of many morphological, physiological, and biochemical characteristics that make up drought resistance (Sullivan and Ross 1979). According to Kramer (1980) and Pallardy (1981) plants can possess more than one type of adaptation. Pallardy *et al.* (1983) also suggested that a strong expression of one mechanism, such as deep rooting, does not preclude the presence of other mechanisms such as osmotic adjustment.

Levitt (1985) reported that the presence of the three major drought resistance mechanisms (drought avoidance, dehydration avoidance, and dehydration tolerance) in cabbage (*Brassica oleracea* var. *capitata*, Early Jersey Wakefield) depended on leaf age. Maximum drought avoidance by cuticular control of water loss occurred in intermediate leaves and maximum dehydration tolerance occurred in upper (younger) leaves of cabbage. Older leaves were not able to acclimate and had no adequate drought avoidance. Drought-induced acclimation increased dehydration avoidance and tolerance but not drought avoidance. This was assumed to be due to

the presence of an already efficient cuticular control on water loss in the non-acclimated leaves. Thus Levitt (1985) recommended hardening plants by means of slow drought stress comparable to that in the field before attempting to determine drought tolerance of a plant in the field. Shcherbakova and Kacperska-Palacz (1980) also found dehydration pretreatment partly protected winter rape (*Brassica napus* L. var. *oleifera* cv. Gorczanski) hypocotyl tissues against injuries from severe desiccation.

Drought tolerance of some plants tends to be greatest during early seedling development and declines in later stages (Clarke and Durley 1981). Greater drought tolerance during the seedling stage is considered important for the establishment of plants in arid areas, while avoidance mechanisms such as extensive root systems make up for a decline in tolerance as the plant grows older.

One drought resistance mechanism is osmotic adjustment, a lowering of osmotic potential due to accumulation of solutes, which in turn lowers the water potential at which stomata close (Turner 1979). Turgor can then be maintained despite a decrease in leaf water potential and water content. Plants that adjust osmotically must be able to tolerate low water potential without damage to tissues (Turner and Begg 1981). A slower rate of drying, greater degree of stress, lower temperature, and higher light level generally produce a higher degree of osmotic adjustment (Turner and Jones 1980).

Solutes involved in osmotic adjustment can be sugars, organic acids, free amino acids, and potassium ions (K^+) (Barlow *et al.* 1980, Turner and Jones 1980). Turner (1979) found that soluble sugar concentration increased when osmotic

adjustment occurred in sorghum and sunflower (*Helianthus annuus* L.). Timpa *et al.* (1986) studied effects of water stress on organic acid and carbohydrate composition of cotton (*Gossypium hirsutum* L.) plants. Organic acids and carbohydrates increased in water stressed plants, however, organic acid increases were greater in drought resistant varieties while total carbohydrates increased more in less resistant types. In a study on drought responses of apical meristems of wheat (*Triticum aestivum* L.), Barlow *et al.* (1980) found increased accumulation of soluble sugars and free amino acids (mainly proline and asparagine) in apices and expanding leaves enclosed in older leaf sheaths. However, solute accumulation in mature, exposed leaves was much lower.

Santarius (1973) postulated that sugars help in the development of stress resistance by stabilizing proteins and therefore membranes against heat, freezing, and water stress by two possible mechanisms: 1) sugars help reduce the concentration of toxic solutes during freezing and prevent membrane inactivation due to their neutral and non-toxic nature, and 2) sugars stabilize membranes against stress by influencing water binding with their $[OH]^+$ groups. Sugars can replace bound water or some phase of water adjacent to sensitive proteins or can be bound directly to proteins. Lee-Stadelmann and Stadelmann (1979) stated that sugars may be directly involved in the development of drought resistance by their association with membrane phospholipids and proteins and replacing water removed by dehydration. Chen and Li (1977) reported an increase in soluble sugars in stem cortical tissues of water stressed red-osier dogwood (*Cornus stolonifera* Michx.). The authors suggested that the sugars may play a protective role in stressed tissues. The

protective role of sugars has also been described by Parker (1972) and Heber and Santarius (1976). In a study on desiccation sensitivity of corn (*Zea mays* L.) and *Pennisetum typhoides* pollen, Hoekstra *et al.* (1989) suggested that the presence of sucrose was a key factor in preserving membranes in dry pollen. They found little difference in the composition and content of phospholipids before and after drying while there was an increase in sucrose content.

III. EFFECTS OF DEHYDRATION ON CELLULAR COMPONENTS AND STRUCTURE

A. Membrane Lipids

Approximately half of the dry weight of plant plasma membranes is lipid (Harwood and Russell 1984). Included among the major components are phospholipids (up to 65%), glycolipids (up to 20%), sterols (up to 5%), and neutral lipids. Both phospholipids and glycolipids are polar acyl lipids and are mainly associated with membranous structures in plant cells (Hitchcock 1975). Phospholipids and proteins are arranged in membranes in a formation described by the "fluid mosaic model" (Salisbury and Ross 1985). According to this model, amphiphilic lipids form a fluid bilayer where nonpolar hydrocarbon tails point inward toward one another while polar heads are on the outside, interacting with the aqueous phases inside and outside the region enclosed by the membrane (Fuller and Nes 1987). Integral proteins are bound tightly within the membrane and peripheral proteins are loosely attached to one or the other side of the membrane surface (Harwood and Russell 1984, Salisbury and Ross 1985). Thus membranes are

asymmetric in regard to the integral membrane proteins (IMP)(Thomson *et al.* 1987). Stability and the selective properties of membranes are believed to be due to the bilayer configuration and incorporation of other compounds such as sterols, proteins, and lipopolysaccharides (Simon 1978b, Salisbury and Ross 1985, Fuller and Nes 1987). Among the lipids involved in the bilayer structure are phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl serine, sphingophospholipids, and digalactosyl diacylglycerol, (Demel 1987).

Chetal *et al.* (1982) reported a decrease in total glycolipids in water-stressed leaves of wheat and barley (*Hordeum vulgare* L.). Although all plants showed an increase in glycolipids upon rewatering, the increase was higher in varieties requiring less water than sensitive varieties. Since glycolipids are the major constituents of chloroplast membranes (Pham Thi *et al.* 1982, Salisbury and Ross 1985, Fuller and Nes 1987), such a decrease will possibly affect photosynthetic rate (Chetal *et al.* 1982).

An increase in phospholipids for water stressed wheat and barley leaves was reported by Chetal *et al.* (1980). The phospholipid increase was greater in drought resistant varieties. An increase in phospholipids is considered an adaptation mechanism to prevent membrane damage due to drought (Chetal *et al.* 1980, Harwood 1983). Huitema *et al.* (1982) also reported increased phospholipid content in drought stressed leaves of wheat, as well as an increased PC/PE ratio. Since an increased PC/PE ratio was found to correlate with high frost resistance, it was proposed that an increased level of PC in the membrane might result in a higher degree of fluidity, a property considered important for survival at low

temperatures (Huitema *et al.* 1982) and dry conditions (Vigh *et al.* 1986). Besides higher phospholipid levels and more unsaturated phospholipids, Horvath *et al.* (1983) reported lowered sterol/phospholipid ratio for cold hardened cucumber (*Cucumis sativus*) leaves. Vigh *et al.* (1986) found increased PC/PE ratio and increased unsaturation of lipids in water stressed wheat leaves. They believed these increases to be adaptive changes which can increase overall membrane fluidity and preserve the membrane bilayer under stress. Pham Thi *et al.* (1982) however, reported a decrease in the phospholipid content of stressed cotton leaves. A drought hardening treatment induced a slight increase in phospholipids, but only in a drought resistant variety.

Increased unsaturation and decreased acyl chain length increases passive permeability of natural and synthetic membranes (Harwood and Russell 1984). Radunz (1987) reported that a high content of unsaturated fatty acids in lipids increased fluidity of biomembranes, which allowed improved ion transport between cell compartments. The increased fluidity changed the activity of phospholipid-dependent membrane-bound enzyme systems. Adler and Liljenberg (1981) suggested that the unsaturation of phospholipids was a way of retaining functionally correct membrane fluidity under salt stress. In their study of salt-tolerance of yeasts, they reported decreased phospholipid unsaturation for non-tolerant species under high salinity. A salt-tolerant yeast (*Debaryomyces hansenii* (Zpf) van Rij) showed more restricted permeability at high salinity than the non-tolerant one (*Saccharomyces cervisial* Hansen). Sterols increased in yeasts exposed to high salinity regardless of their salt tolerance. Adler and Liljenberg (1981) found that the molar ratio of free

sterols to phospholipids was higher in *D. hansenii* than in *S. cervisial* irrespective of growth medium salinity. Sterols are believed to have a stabilizing effect on biological membranes (Hendrix and Higinbotham 1973). This role was shown by Grunwald (1968) in a study of alcohol-treated red beet (*Beta vulgaris* L.) roots, where sterols reversed the increased membrane permeability (leakage of red pigment) caused by the alcohol. Free sterols were effective in stabilizing leakage by penetrating the phospholipids of the membranes.

B. Membrane Proteins

According to Saxton *et al.* (1980) the primary effect of drought on membranes is a loss of turgor pressure, changing the mechanical stress on the membrane and its interaction with the cell wall. They suggest that a pressure-sensitive lateral phase separation affects either diffusion and interaction of mobile membrane proteins, or the conformation of a membrane protein, or both. Bewley and Krochko (1982) believe that proteins play a role in solute leakage control. They found that slow desiccation of the moss *Tortula ruralis* (Hedw.) Gaertn, Meyer, and Scherb induced more changes in phospholipid composition than rapid desiccation, despite more leakage upon rehydration from rapid desiccation. Due to the lack of correlation between the extent of leakage during rehydration and changes in fatty acid composition, they suggested that the phospholipid component of the membrane played an insignificant role in leakage control and suspected a role for proteins instead.

Water stress reduces protein synthesis in drought sensitive tissues of many plants (Bewley 1979, Clarke and Durley 1981). However, cytoplasmic proteins are more stable to denaturation, coagulation, or hydrolysis in drought resistant plants (Sullivan and Eastin 1974). Brandle *et al.* (1977) reported that protein synthesis of black locust (*Robinia pseudoacacia* L.) was resistant to water stress despite some decline. They found increased ribonuclease (RNase) activity with increased water stress. Dhindsa and Bewley (1976) and Bewley and Krochko (1982) also reported increased activity of RNase and loss of polysomes in water stressed moss. However, there was no correlation between the increased RNase activity and decline in polysomes. Valluri *et al.* (1988) reported that new proteins were induced by mild water stress in slash pine (*Pinus elliottii* Engelm.) callus cultures but the role of these proteins in drought tolerance is not known.

Thind and Malik (1988) reported synthesis of new amino acids which were not detected in nonstressed wheat plants. When 3-day-old seedlings were exposed to -0.4 MPa osmotic stress (using poly-ethylene glycol-6000 solutions), two heterocyclic amino acids were found which were not observed in the control. They found more than double the concentrations of leucine and isoleucine, four times the alanine, and a six-fold increase in proline in stressed as compared to nonstressed control plants. An increase was also observed in glutamine and asparagine while there was a decrease in aspartic acid. They concluded that the accumulation of amino acids was due to both *de novo* synthesis and hydrolysis of proteins.

Shen *et al.* (1989) reported increased concentrations of some free amino acids in drought stressed (soil water potential of -1.2 MPa) flatpea (*Lathyrus*

sylvestris L.) depending on plant age and tissue type (leaf, root, or stem). The concentrations of valine, isoleucine, leucine, and phenylalanine were each 3.5, 3.5 and 2.3 times higher than control plants for stems, roots, and leaves respectively. Concentrations of asparagine, arginine, glutamine, and aspartic acid (all acidic amino acids) were not influenced by water status. There was a general increase in free amino acids with plant age over the 14-week observation. Proline increased in all stressed tissues and was correlated with plant age. It represented about 10% of the total amino acids quantified. They suggested that the individual amino acids observed might have some specific function in growth and developmental processes, in stress tolerance, or in stress adaptations.

C. Solute Leakage and Cell Membrane Configuration

A discussion of solute leakage from dry seeds upon rewetting may help explain leakage from other tissues upon rehydration after drought. There is disagreement as to why dry seeds have a high rate of solute leakage during the first 2 minutes of imbibition that declines rapidly to a lower rate after about 5 minutes (Larson 1968, Simon 1974, McKersie and Stinson 1980, Powell and Matthews 1981). A similar phenomenon has also been shown by Shcherbakova and Kacperska (1983) in winter rape hypocotyls where rapid leakage occurred during the first 4 hours of rehydration followed by a slower but continuing leakage.

According to Larson (1968), the initial high rate of solute leakage in imbibing seeds is due to membrane damage caused by rapid imbibition. Powell and Matthews (1981) proposed another hypothesis to explain the time course of leakage.

In their study of leakage rates from living and dead pea (*Pisum sativum* L.) seeds, they found similar patterns of solute loss, although the amount was higher for dead seeds. They suggested that the leakage was a result of a physical diffusion phenomenon. Their explanation for initial high leakage, a rapid loss of solutes from the outer layers, was in agreement with that of Simon (1974, 1978a). However, they explained the decline with time differently. According to Powell and Matthews (1981) it was due to a longer diffusion path for solutes from the inner layers. The decrease in total leakage with repeated drying/imbibing cycles was explained by a decrease in the amount of leachates present. Simon's (1974, 1978a) explanation was that the interior cells become hydrated at a later stage as the water has to pass through many cell layers and, in the process, restoration of membrane integrity occurs and reduces leakage. Powell and Matthews (1981) argue that this mechanism fails to explain the observation on dead seeds, where restoration of membrane integrity is not expected.

At low water content (20% or less), Simon (1974) suggested that there are changes in orientation of phospholipids and membrane proteins. A transition was proposed from the normal, lamellar bilayer configuration to a tubular, hexagonal$_{II}$ (H_{II}) phase to explain solute leakage with dehydration. Lipids with a relatively small polarity such as unsaturated PE are reported to favor the formation of a H_{II} phase (Harwood and Russell 1984, Demel 1987). In the H_{II} phase, polar head groups form long cylinders oriented into an aqueous core (Simon 1974, 1978b). In such transitions, a change in the semipermeable properties of the membrane may occur. The phospholipids are expected to change from the H_{II} to the lamellar

configuration upon rewetting. During the period between the H_{II} and lamellar configurations, the membrane will be disorganized and freely permeable. Since the cytoplasm rehydrates while membrane integrity is being reestablished, solutes leak out of the cells (Simon 1974, 1978a).

The phase transition hypothesis is challenged based on freeze-fracture and X-ray diffraction studies. Platt-Aloia (1986) and Thomson *et al.* (1987) reported a bilayer organization of all membranes and no H_{II} phase in dry seeds and pollen of different species. This was despite differences in water content ranging from 12% to 55% before imbibition. After a study on *Lotus corniculatus* L. seed membranes by X-ray diffraction, McKersie and Stinson (1980) found no hexagonal phase formation by membrane phospholipids.

Increased incidence of cell rupture and altered membrane permeability in dehydrated soybean (*Glycine max* L. Merr.) axes after 36 hours of imbibition were reported by Senaratna and McKersie (1983). They found that solutes that leaked from axes between 2 and 8 hours of rehydration did not originate from ruptured cells. Their conclusion was that dehydration stress may have increased permeability by either increasing the rate of passive diffusion across the phospholipid bilayer or decreasing the rate of active uptake. The increase in passive diffusion was assumed to be due to a reduction in integrity of the lipid bilayer.

Complete structural disruption of the tonoplast of maize leaves was reported by Giles *et al.* (1976) at a leaf water potential of -1.8 MPa. Chloroplasts swelled and burst upon coming into contact with the vacuolar fluid. For sorghum leaves in the same study, no changes were observed in the tonoplast or outer chloroplast

membrane at a leaf water potential of -2.5 MPa. In their most severely stressed plants (leaf water potential -3.7 MPa), sorghum tonoplasts appeared to have fragmented to many small vesicles while the only apparent chloroplast damage was the swelling of the outer membrane. From these findings they concluded that maintenance of tonoplast integrity is an important factor in the ability of plants to withstand drought.

Fellows and Boyer (1978) reported that cells of sunflower leaves desiccated to a leaf water potential of -1.5 MPa showed minor differences from non-stressed controls, while further desiccation had different effects depending on the organelle of interest. Lipid droplets and associated vesicles were observed frequently in the cytoplasm. Tonoplast and plasmalemma breakdown appeared more often with desiccation to -2.2 MPa. Massive wall infolding, contraction of the vacuole, concentration of the cytoplasm, cell wall-plasmalemma separation, an increase in the number of cells showing breakage of the plasmalemma and/or tonoplast, and major disintegration of the cell contents (40% of all cells) were observed at a more severe desiccation level of -2.6 MPa. Despite these changes, chloroplasts, mitochondria, and nuclei appeared intact.

IV. SOLUTE LEAKAGE AND WATER STRESS

Whether due to membrane damage, phase transition of lipids, or simple physical diffusion phenomena, there is ample evidence that loss of solutes due to stress-induced membrane leakage can affect growth (Larson 1968) or survival (Dlugokecka and Kacperska-Palacz 1978). Among the solutes that leak out are:

amino acids, sugars, organic acids, hormones, phenolics, phosphates, various fluorescent materials, and electrolytes such as K^+ ions (Palta *et al.* 1977, Bewley 1979, Shcherbakova and Kacperska 1983). Disorganization of membrane structure and increased permeability may be due to factors common to different types of stress including nutritional status, soil acidity, fungal infection (Harwood 1983), flooding (Shcherbakova and Kacperska 1983), bacterial infection (Hanker and Kudelova 1986), and air pollution (Navari-Izzo *et al.* 1989).

Leakage of electrolytes has been used as an indication of cell injury and tolerance of stress due to heat (Sullivan and Ross 1979), freezing (Flint *et al.* 1967, Zhang and Willison 1987), salt (Redmann *et al.* 1986), or water stress (Sullivan and Eastin 1974, Matthews and Rogerson 1976, Gupta 1977, Palta *et al.* 1977, Dlugokecka and Kacperska-Palacz 1978, Simon 1978a, Sullivan and Ross 1979, Bewley 1979, Blum and Ebercon 1981, Leopold *et al.* 1981, Krishnamani *et al.* 1984, Martin *et al.* 1987). To avoid variability among samples due to differences in total electrolytes, comparisons of leakage are made by calculating what Flint *et al.* (1967) called an "index of injury". The injury index is a scale based on a zero value for a sample with no leakage and a value of 100 after the same sample is autoclaved. Flint *et al.* (1967) calculated an injury index to quantify electrolytic leakage due to freezing and a similar method was used in dehydration studies by Dlugokecka and Kacperska-Palacz (1978). They reported comparable results in injury index calculations based on either survival percentage or electrolyte leakage. The assumption made in using conductivity or UV-absorbance as a measure of stress injury is that the longer it takes for membrane reconstruction or membrane

stability to occur, the higher the injury index and the less stress tolerant the plant is (Flint *et al.* 1967, Gupta 1977, Simon 1978b, Dlugokecka and Kacperska-Palacz 1978, Sullivan and Ross 1979, Bewley 1979, Leopold *et al.* 1981).

Martin *et al.* (1987) used an injury index based on measurement of electrolyte leakage to compare dehydration tolerance of six woody species whose dehydration avoidance was known. Based on stomatal response to drought, Hinckley *et al.* (1979) ranked the dehydration avoidance of these species as follows: *Quercus velutina* Lam. = *Q. alba* L. > *Q. rubra* L. > *Acer saccharum* Marsh. > *Juglans nigra* L.= *Cornus florida* L. Species that had their stomata open for the longest period during drought stress were considered the most drought resistant. At the first sampling date (June) of their dehydration tolerance study, Martin *et al.* (1987) found that the species ranking of tolerance by injury index was similar to the dehydration avoidance ranking. Later in August however, *Cornus florida* L., which was the least resistant early in the season, was found to be one of the most dehydration tolerant species. The injury index ranking for dehydration tolerance obtained late in the season (July) (least injured to most injured) was as follows: *Q. rubra* = *Q. velutina* = *Q. alba* = *C. florida* > *A. saccharum* > *J. nigra.* According to this and an earlier study by Hinckley *et al.* (1979), both mechanisms of drought tolerance, dehydration avoidance and dehydration tolerance, were observed for the *Quercus* species. Since there was drought and a high temperature period during the months of July and August, Martin *et al.* (1987) concluded that dehydration tolerance of all species except black walnut improved due to drought hardening.

V. WATER RELATIONS OF *POPULUS DELTOIDES*

Regehr *et al.* (1975) found photosynthesis in *P. deltoides* to be very sensitive to drought. It decreased sharply when leaf water potential was below -0.85 MPa and reached zero at -1.1 MPa. They also tested the degree of recovery of photosynthesis after water stress. Complete recovery required 36 hours at an initial leaf water potential of -1.05 MPa, while it required 46 hours to reach 50% of the maximum rate after rehydration from -1.55 MPa. Scarascia-Mugnozza *et al.* (1986) reported zero net photosynthesis and leaf conductance below a leaf water potential of -2.5 MPa. After examining leaf epidermal strips, Schulte and Hinckley (1987) reported plasmolysis of *P. deltoides* guard cells at about -2.0 MPa followed by stomatal closure.

In a study of clones of eastern cottonwood from dry and wet sites, Kelliher and Tauer (1980) found that dry site clones had lower stomatal resistance values than wet site plants even under well watered conditions. For selection purposes, they recommended a clone which can tolerate drought stress with the least increase in stomatal resistance and grow the fastest or continue to grow the longest under drought.

Coleman (1982) investigated the drought resistance of *P. deltoides* clones from Missouri ("Mighty-Mo"), Nebraska ("Platte"), and Ohio ("Ohio Red"). These clones were selected on the basis of their superior growth characteristics. Platte was considered the most drought tolerant (avoiding) clone, since it had a faster growth rate, lower transpiration, and the largest root/shoot ratio observed. Ohio Red, which had the highest predawn xylem pressure potential, lowest leaf area, and

highest bound water, was shown to have good drought resistance properties. Mighty-Mo was intermediate in most parameters.

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EXPERIMENT I:

SEASONAL AND CLONAL VARIATIONS IN WATER RELATIONS OF *POPULUS DELTOIDES*

ABSTRACT

Drought resistance of five field-grown eastern cottonwood (*Populus deltoides* Bartr.) clones was examined during the 1988 and 1989 growing seasons. Clonal and seasonal variations in leaf water potential (Ψ_w), leaf osmotic potential (Ψ_s), dry weight fraction (DWF), and height growth were investigated. During dry periods, DWF increased and predawn Ψ_s declined to as low as -2.1 MPa from a high of -1.4 MPa. There were significant negative correlation coefficients between DWF and Ψ_s for most clones. High predawn Ψ_w values were found for clones from Nebraska ("Platte") and Missouri ("Mighty Mo") in 1988. In "Tippecanoe" (Indiana clone), predawn Ψ_s values were consistently lower and significantly different from those of Platte for most dates in 1988. There were no differences between Platte and Tippecanoe in Ψ_s for most 1989 sample dates and both had lower values than a clone from Ohio ("Ohio Red"). Ohio Red showed the lowest height growth in 1988. No significant height differences were found among clones after June 1989.

Since all clones had lower Ψ_s and higher DWF during dry periods, it was concluded that all clones have some degree of drought tolerance. Platte and Tippecanoe were the most drought tolerant clones.

INTRODUCTION

Plants differ in their sensitivity and response to drought stress. The degree to which a plant withstands drought stress is known as drought resistance (Jones *et al.* 1981). Drought resistance can be classified as either drought avoidance or drought tolerance. Drought avoiding plants maintain a high tissue water potential by reducing water loss or maintaining water uptake, while drought tolerant plants have the ability to withstand water stress (Levitt 1980). Since the extent of drought avoidance is limited to short and mild dry periods, plants must be drought tolerant to grow and survive in areas where drought is more common (Kramer 1983).

Clonal variations in drought resistance of eastern cottonwood (*Populus deltoides* Bartr.) have been reported (Kelliher and Tauer 1980, Pallardy and Kozlowski 1981). Regehr *et al.* (1975) found *P. deltoides* plants from Minnesota were sensitive to water stress. Net photosynthesis dropped sharply below a leaf water potential of -0.8 MPa and reached zero at -1.1 MPa. However, Scarascia-Mugnozza *et al.* (1986) found that net photosynthesis of *P. deltoides* from Mississippi did not reach zero until a leaf water potential of -2.5 MPa was attained. Coleman (1982) also reported clonal differences in predawn xylem pressure potential of *P. deltoides* during a 13 day stress period.

The lowering of osmotic potential due to solute accumulation, osmotic adjustment, is one characteristic of drought tolerant plants (Levitt 1980, Jones *et al.* 1981). Plants that adjust osmotically can withstand mild water stress and continue to have turgid cells. Declines in osmotic potential with season are known to occur in many tree species (Abrams 1988) including *P. deltoides* (Tyree *et al.* 1978).

A high dry weight to turgid weight ratio of leaf laminas, known as dry weight fraction (Hellkvist *et al.* 1974, Parker and Pallardy 1987), has been related to drought tolerance of several species including Sitka spruce (*Picea sitchensis* (Bong.) Carr.)(Hellkvist *et al.* 1974), cotton (*Gossypium hirsutum* L.)(Cutler and Rains 1978), *Macroptilium atropurpureum* (Wilson *et al.* 1980), and perennial ryegrass (*Lolium perenne* L.)(Thomas 1987).

The purpose of this study was to compare seasonal and clonal variations in leaf water potential, leaf osmotic potential, and dry weight fraction of five *Populus deltoides* clones from different parts of the species' range grown under field conditions.

MATERIALS AND METHODS

Plant Material

Five *Populus deltoides* Bartr. clones were selected from a provenance plantation established in 1966 at the University of Nebraska's Agricultural Research and Development Center near Mead, Nebraska (Ying and Bagley 1976). The clones were from Nebraska (36-5), Missouri (251-3), Ohio (217-1), Indiana (44-3), and Illinois (264-4)(numbers designate the original provenance source)(Figure 1). The first three, "Platte", "Mighty-Mo", and "Ohio Red" respectively, were chosen for high growth rates and because some aspects of their water relations had already been described (Coleman 1982). The Indiana clone, known here as "Tippecanoe", was chosen for its similarity to Platte, Ohio Red, and Mighty Mo in growth rate (Table 1). The Illinois clone, known here as "Rock Island", was selected for its

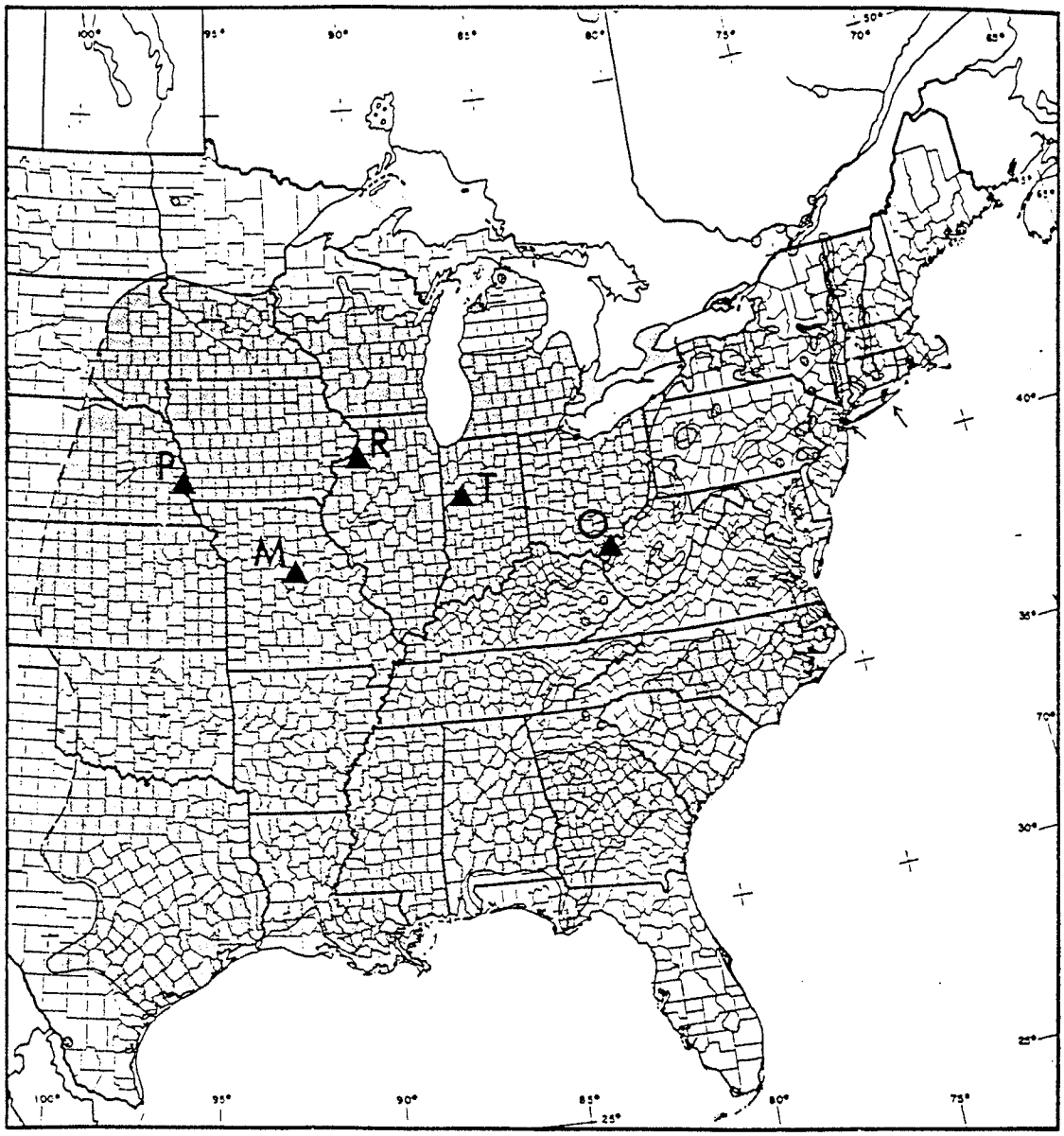


Figure 1. Natural range of *Populus deltoides* Bartr. (shaded) and location of five selected clones (▲) (M- Mighty Mo, P- Platte, O- Ohio Red, R- Rock Island, and T- Tippecanoe). From Silvics of Forest Trees of the United States (H.A. Fowells, USDA Agric. Handbook 271, 1965).

slow growth rate. Average diameters of the clones for 1973, 1978, and 1988 are shown in Table 1 (unpublished data, Department of Forestry, Fisheries, and Wildlife).

Table 1. Average diameter (cm) of five provenances at three measurement years. All plants were established from unrooted cuttings in 1966. Population standard deviations are shown in parentheses (n = number of trees)(unpublished data, Department of Forestry, Fisheries, and Wildlife).

Provenance	n	Year 1973	1978	1988
Mighty Mo	2	22.23 (0.180)	28.96 (0.718)	37.08 (1.437)
Ohio Red	2	20.83 (4.311)	29.21 (2.514)	37.97 (2.694)
Platte	3	21.84 (1.164)	31.41 (4.082)	42.33 (5.232)
Rock Island	3	14.82 (3.595)	20.15 (4.130)	25.82 (5.281)
Tippecanoe	3	20.91 (1.729)	29.38 (3.613)	38.78 (6.674)

Tippecanoe and Rock Island cuttings (23 cm long) were collected from a stool bed on East Campus at the University of Nebraska in Lincoln (UNL) on March 4, 1988. Cuttings of Platte, Mighty-Mo, and Ohio Red were collected from a stool bed at Bessey Nursery (USDA Forest Service) at Halsey, Nebraska (41°54' N, 100°19' W) on February 29, 1988. All cuttings were moistened and stored in plastic bags at 4°C until planting.

On March 16, 1988, forty cuttings of each clone were planted 16.5 cm deep in 946 ml milk containers with a steam-sterilized potting mixture of vermiculite, peat, and silty clay loam soil (1:1:1). These potted cuttings were placed in a greenhouse at UNL. They were watered to saturation every other day until April 15, 1988, and less often thereafter. The rooted cuttings (187) were moved to a

shade house on May 6, 1988. They were randomly planted on a 1 m by 1 m spacing in an 8 m by 20 m plot on the UNL campus on May 12, 1988. The plot was fenced with wire mesh to prevent rodent damage. Plants were sprayed with malathion three times during the first growing season to reduce leaf damage by aphids and other insects. Weeds were controlled by manual cultivation and with directed sprays of glyphosate. Plants were watered from planting date until May 30, 1988 to aid establishment. Plants were also watered occasionally due to hot and dry weather and to reduce damage by a stem canker which ultimately killed 74 plants. Watering was discontinued after August 10, 1988 when plants received precipitation and had sufficient number of leaves for the study.

All five clones were studied in 1988, but only three clones were studied in 1989. Ohio Red was selected for its low predawn water and osmotic potentials and low height growth in 1988. Tippecanoe was selected because it had similar water relations to Ohio Red but grew faster. Platte was selected for its high predawn leaf water and osmotic potentials and its fast growth.

Environmental Measurements

Daily precipitation values and air temperatures were obtained from an automated weather station located 150 m east of the planting. Irrigation amounts (in 1988) were estimated by measuring the average depth of water collected in six randomly placed beakers on the plot.

Plant Measurements

The date of bud break for each cutting was recorded when cuttings were in the greenhouse. Height was measured from May through September of 1988 and May through August of 1989. The first measurement in 1988 was taken while plants were in the shade-house a week before transplanting (Table 2).

Leaf water potential (Ψ_w) and leaf osmotic potential (Ψ_s) were measured on separate leaves with a pressure chamber (Ritchie and Hinckley 1975, Turner 1981) and thermocouple psychrometer (Decagon Devices Inc., Pullman, WA), respectively. Predawn Ψ_w and predawn Ψ_s were measured every two to three weeks from June to September 1988 (Table 2) on a random sample of fully developed leaves from three plants per clone (one leaf per plant for a total of 15 leaves each for Ψ_w and Ψ_s). All leaves sampled were randomly taken from between the fourth and eighth of fully developed leaves counting from the apex. At each sampling date, both Ψ_w and Ψ_s were measured from the same plant when plants had an adequate number of leaves. Solar noon Ψ_w and Ψ_s of three plants per clone were measured on June 14 and June 28, 1988. These measurements were discontinued due to low growth rates and a lack of available leaves.

Osmotic potential of three rehydrated leaves per clone were also measured four times in 1988 (Table 2). Three leaves were collected at predawn and the petiole was immediately placed in a test tube filled with water. They were brought to a laboratory covered with a plastic bag and allowed to rehydrate for 24 h in the dark at room temperature before Ψ_s was measured.

Table 2. Measurements taken on each sampling date during 1988 and 1989 growing seasons.

Sampling date	Type of Measurement: Leaf water and osmotic potentials	Others
1988		
5/07	-	Height
6/07	-	Height
6/14	Predawn and solar noon	
6/21	Predawn and rehydrated	
6/28	Predawn and solar noon	
7/11	-	Height
7/19	Predawn and rehydrated	
8/02	Predawn	
8/10	-	Height
8/16	Predawn and rehydrated	
8/30	Predawn	
9/13	Predawn and rehydrated	
9/19	-	Height
9/27	Predawn	
1989		
5/11	-	Height
5/30	Predawn	
6/11	-	Height
6/13	Predawn and rehydrated	
6/27	Predawn and rehydrated	
7/11	Predawn and rehydrated	
7/12	-	Height
7/28	Predawn and rehydrated	
8/09	Predawn	Height
8/21	Predawn and rehydrated	

From each plant selected for Ψ_s measurement (predawn, solar noon, and rehydrated), a 12 mm by 45 mm strip was cut from a leaf and wrapped around the inner wall of a thermocouple psychrometer sample cup. A circular piece of leaf with a diameter of 22 mm was cut to cover the bottom of the cup which was then sealed with tape, labelled, and immediately placed on dry ice or in a freezer.

Microvolt readings from the thermocouple psychrometer were made with NT-3 nanovoltmeter-thermometer (SC-10A, Decagon Devices Inc., Pullman, WA). Two or three potassium chloride standards were included at each equilibration and were used to calculate a psychrometer constant. Psychrometer values were converted to Ψ_s using this constant.

Relative water content (RWC) and dry weight fraction (DWF) were measured for each leaf sampled for predawn and solar noon Ψ_w readings. Ten 1-cm-diameter discs were sampled from each leaf with a cork borer and their fresh weight (FWT) was measured to 0.1 mg with an analytical balance. The weighed discs were floated on water in covered petri dishes for 8 h at room temperature and ambient light (Barrs 1968). The discs were then gently blotted with tissue paper and turgid weight (TWT) was determined. They were oven-dried at 85°C for at least 6 h, and weighed again (dry weight--DWT). Relative water content and DWF were calculated from:

RWC=100(FWT-DWT)/(TWT-DWT) (Barrs 1968)

DWF=DWT/TWT (Hellkvist *et al.* 1974).

The number of plants sampled per clone for Ψ_w, Ψ_s, and RWC was increased from three to seven in 1989. On each of the seven sample dates from May to August, predawn Ψ_w and Ψ_s measurements were made on separate leaves from the same plant. Some plants were sampled on each sample date (i.e. seven times) because of the limited number of plants available (e.g. 10 for Ohio Red). Leaves sampled for rehydrated Ψ_s in 1989 (measured five times, from June to August),

were collected from the same plants that were sampled for predawn Ψ_s and Ψ_w. The rehydration time was reduced from 24 to 13 h because leaves reached about the same saturation level (about -0.1 MPa) after 13 h as after a 24 h period. No Ψ_s was measured on May 30 because of rain at the time of sampling.

Data Analysis

Height, Ψ_w, Ψ_s, RWC, and DWF values were compared for each sample date using Duncan's Multiple Range Test (Steel and Torrie 1980). A clone by sample-date interaction was tested and data analyzed accordingly when significant. The level of significance used to test all differences was $\alpha \leq 0.05$.

RESULTS

Height Growth

Ohio Red showed the lowest average total height and Platte the highest throughout the 1988 growing season (Figure 2). Ohio Red cuttings generally broke dormancy later than other clones. Average heights of Platte, Tippecanoe, and Mighty Mo were not significantly different except in May 1988, when Platte was significantly taller than Mighty Mo. Growth rates (equal to the slope of the lines) for all clones from June to July were lower than May to June growth rates. The highest growth rates for all clones were observed in July-August when there was more irrigation and precipitation (Figure 3d).

Early in the 1989 growing season, only Ohio Red was significantly smaller in height from Platte, however, this difference disappeared in July (Figure 2). The

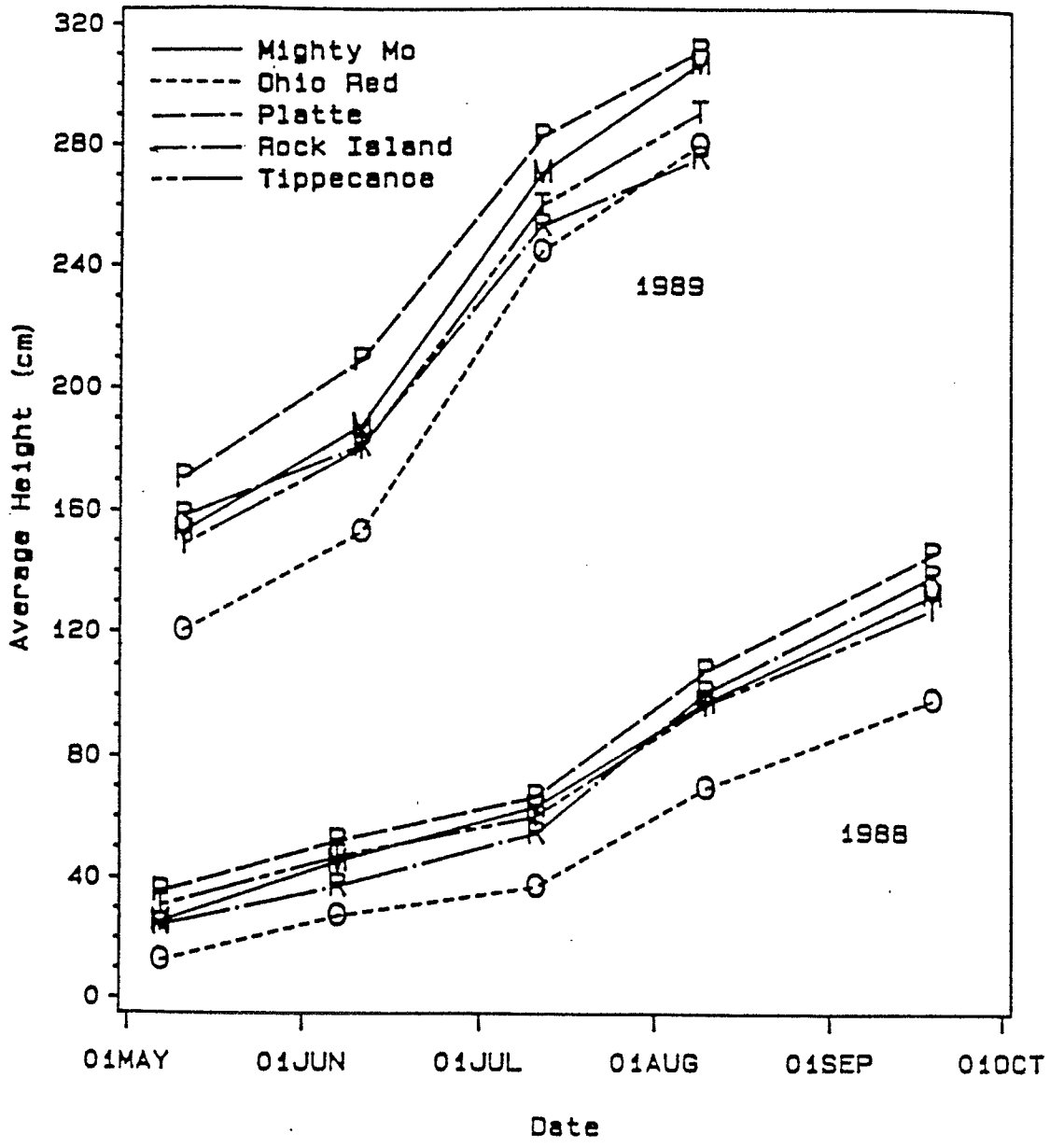


Figure 2. Average height of five *Populus deltoides* clones by date (cm) for 1988 and 1989 (n=9).

highest growth rates for all clones were observed between June and July in conjunction with increased precipitation. Growth rates then declined in the July-August period when precipitation was lower (Figure 5d).

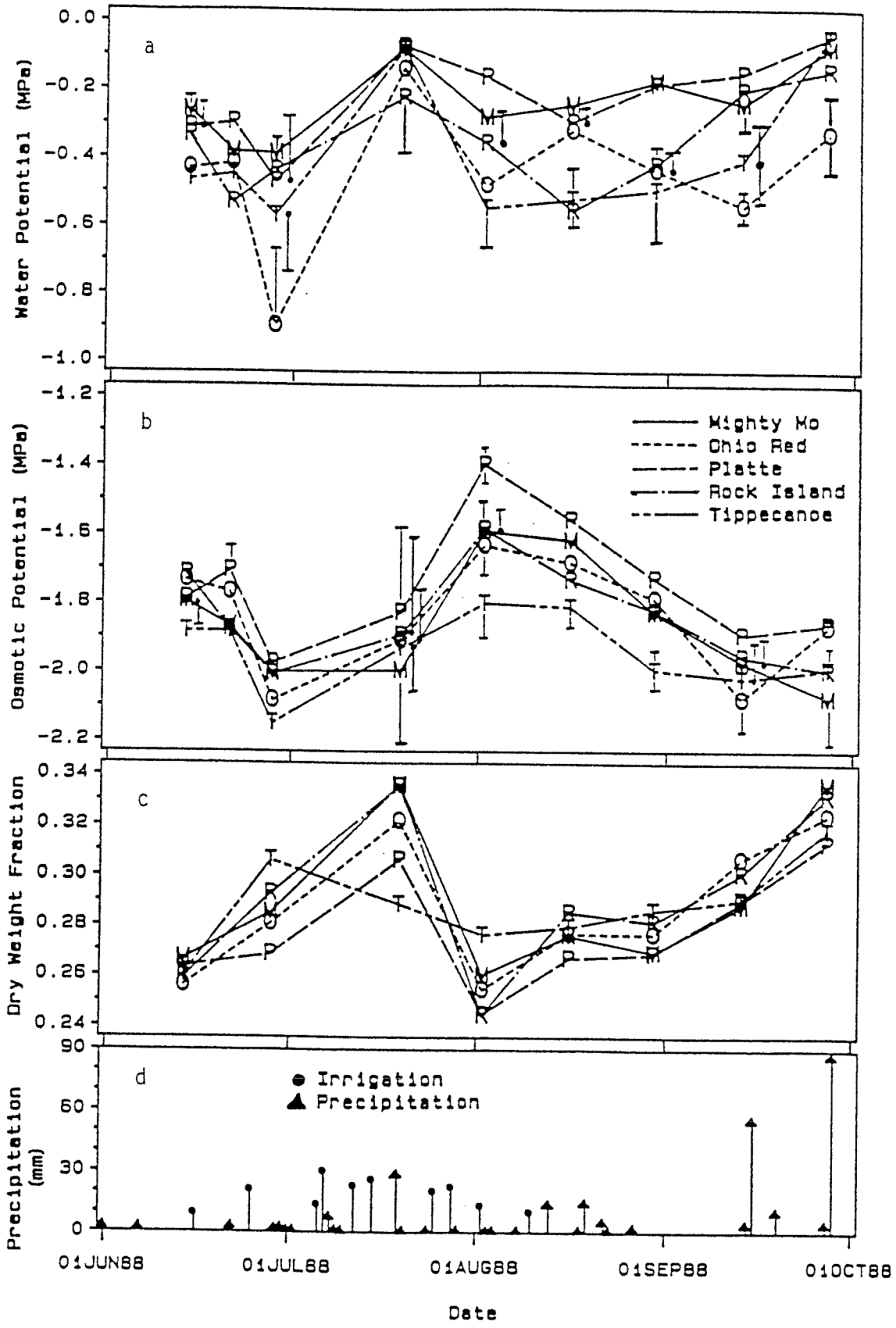
Water Relations

1988 Growing Season

In 1988 most clones had their lowest average predawn Ψ_w and predawn Ψ_s values on June 28 (Figure 3). Significant differences were found among clones in average predawn Ψ_w and predawn Ψ_s for most sample dates. Ohio Red showed lower predawn Ψ_w than Platte on all sample dates with significant differences on June 21, August 2, September 13, and September 27. Platte and Mighty Mo generally had high predawn Ψ_w and were not significantly different at any sample date. Platte had the highest Ψ_s at all dates except on June 14. Predawn Ψ_w and Ψ_s values of Tippecanoe were significantly lower than Platte in August. Significant differences were also found between these two clones on June 21 and September 13 in predawn Ψ_w and on June 28 in predawn Ψ_s. Predawn Ψ_s values of Tippecanoe were low and varied little by date compared to the other clones. This was even more evident in rehydrated leaves (Figure 4b). This pattern was also observed for solar noon Ψ_s (data not shown).

No significant differences were detected in RWC except in August when Rock Island had lower RWC than Mighty Mo and on september 13 when Ohio Red had lower RWC than the other clones (data not shown).

Figure 3. Seasonal and clonal variations in water relations of five *Populus deltoides* clones (n=3) and daily precipitation in 1988. (a) Mean predawn leaf water potential (MPa), (b) predawn osmotic potential (MPa), (c) dry weight fraction, and (d) daily precipitation or irrigation (mm). Standard errors are shown when they exceed the clone's symbol height and may be offset or one sided for clarity. (Precipitation data obtained from Center for Agricultural Meteorology and Climatology, UNL).


a
Water Potential (MPa)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
b
Osmotic Potential (MPa)
-1.2
-1.4
-1.6
-1.8
-2.0
-2.2
Mighty Mo
Ohio Red
Platte
Rock Island
Tippecanoe
c
Dry Weight Fraction
0.34
0.32
0.30
0.28
0.26
0.24
d
Precipitation (mm)
90
60
30
0
Irrigation
Precipitation
01JUN88
01JUL88
01AUG88
01SEP88
01OCT88
Date

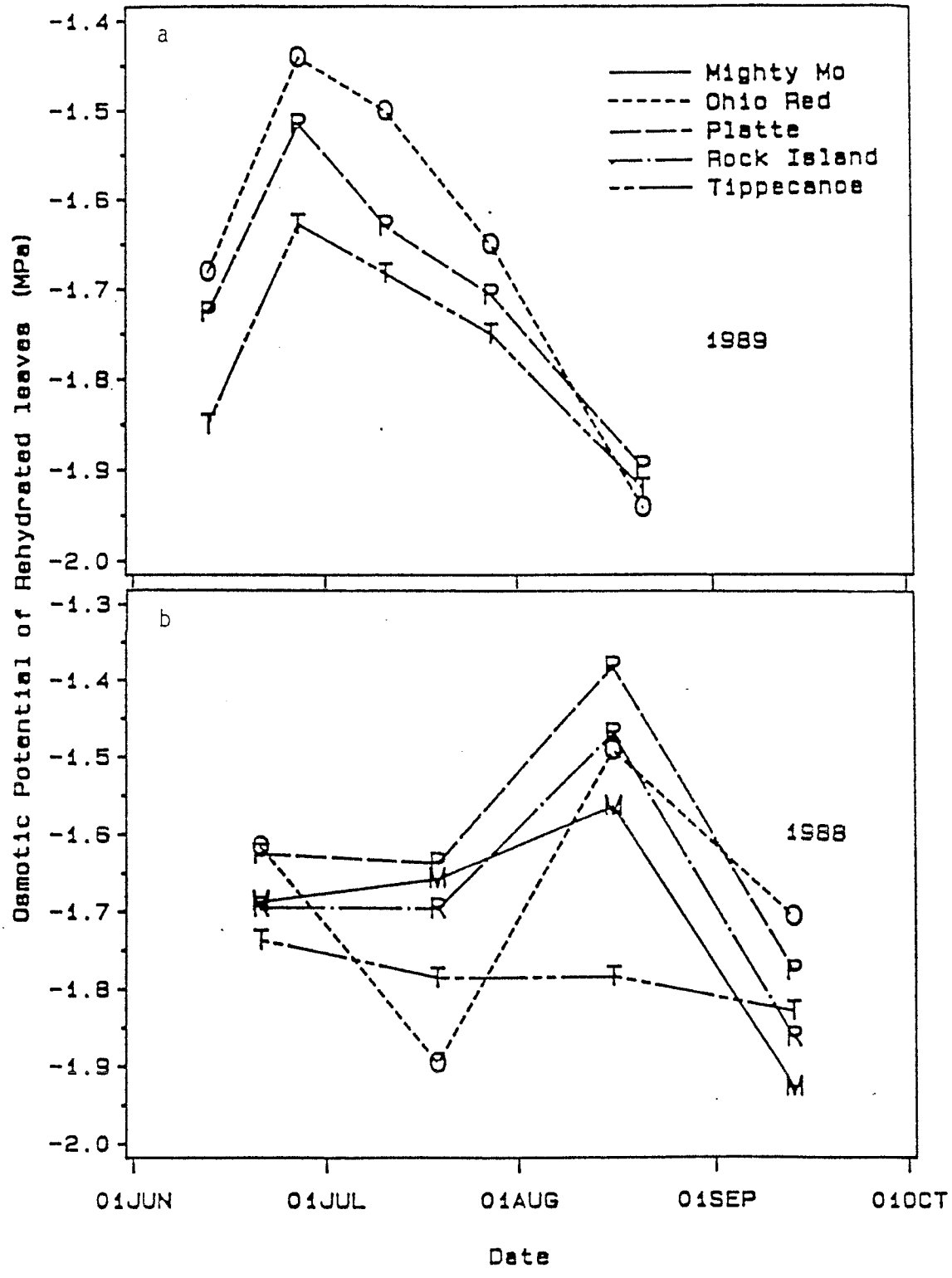


Figure 4. Average osmotic potential (MPa) for rehydrated leaves collected at predawn in (a) 1989 (n=7), and (b) 1988 (n=3).

1989 Growing Season

There was no significant difference in predawn Ψ_w among the three clones for most sample dates (Figure 5a). The only exceptions were on May 30, when Platte had the highest predawn Ψ_w, and on July 11 when Ohio Red had the highest predawn Ψ_w. Ohio Red showed significantly higher predawn Ψ_w than Tippecanoe on July 28. There were no differences between Platte and Tippecanoe in predawn Ψ_s during the season (Figure 5b). Both clones also showed lower predawn Ψ_s than Ohio Red except in August. There were no significant differences in predawn Ψ_s among clones on August 9 while Ohio Red had significantly higher values than Tippecanoe on August 21. Predawn Ψ_w and Ψ_s were the highest of the season on June 27, 1989 for all clones and continued decreasing until the lowest values were obtained in August. The decline in predawn Ψ_s with water stress and season was also evident on rehydrated leaves (Figure 4a). Rehydrated leaves of Tippecanoe had significant lower Ψ_s than those of Ohio Red on all dates except on August 21.

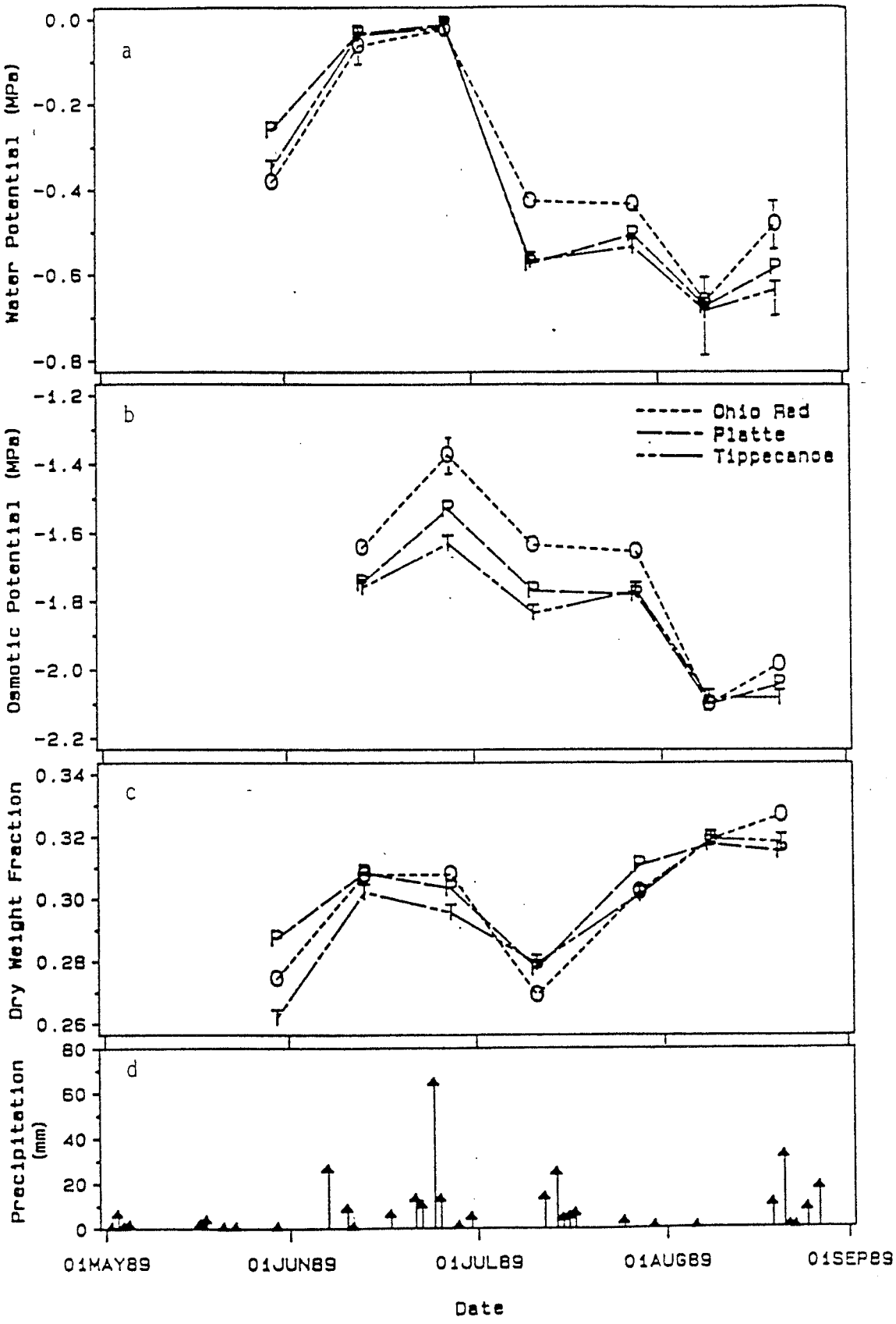
Ohio Red showed significantly higher RWC than Platte and Tippecanoe on June 27, July 28, and August 21, 1989 (data not shown). There were no significant differences among clones on the remaining dates.

Dry Weight Fraction

1988 Growing Season

Dry weight fractions were lowest on June 14 and August 2 (Figure 3c). The highest DWF values were found on July 19 and September 27, for all clones except Tippecanoe. The highest DWF values for Tippecanoe were found on June 28 and

Figure 5. Seasonal and clonal variations in water relations of three *Populus deltoides* clones (n=7) and daily precipitation in 1989. (a) Mean predawn leaf water potential (MPa), (b) predawn osmotic potential (MPa), (c) dry weight fraction, and (d) daily precipitation (mm). Standard errors are shown when they exceed the clone's symbol height and may be offset or one sided for clarity. (Precipitation data obtained from Center for Agricultural Meteorology and Climatology, UNL).


a
b
c
d
Water Potential (MPa)
Osmotic Potential (MPa)
Dry Weight Fraction
Precipitation (mm)
Ohio Red
Platte
Tippecanoe
0.0
-0.2
-0.4
-0.6
-0.8
-1.2
-1.4
-1.6
-1.8
-2.0
-2.2
0.34
0.32
0.30
0.28
0.26
80
60
40
20
0
01MAY89
01JUN89
01JUL89
01AUG89
01SEP89
Date

September 27. Significant differences in DWF among clones were observed on four sample dates, June 28, August 2, August 30, and September 13. On the first three of these dates, Platte had significantly lower DWF than Tippecanoe. On September 13, the DWF values for Tippecanoe and Platte were significantly lower than for Ohio Red. Solar noon DWF values were not significantly different from predawn DWF values for any clone (data not shown). A negative correlation between predawn Ψ_s and DWF was shown for all clones (Table 3).

1989 Growing Season

Significant differences in clone DWF were observed only on May 30, when Platte had the highest DWF and Tippecanoe the lowest (Figure 5c). Ohio Red had the lowest DWF on July 11 and the highest of the season on August 21. Ohio Red was the only clone that showed an increase in DWF from August 9 to August 21. As in 1988, the lowest DWF values were early in the season (May 30) and in mid-summer (July 11). There was a strong negative correlation between predawn Ψ_s and DWF for all clones (Table 3).

Table 3. Correlation coefficients (r) between predawn leaf osmotic potential and dry weight fraction for five *Populus deltoides* clones. Probabilities are shown in parentheses (n = number of leaves).

	Growing Season			
	1988		1989	
Clone	n	r	n	r
Mighty Mo	17	-0.87 (0.0001)	-	-
Ohio Red	12	-0.55 (0.0636)	42	-0.42 (0.0051)
Platte	15	-0.64 (0.0108)	42	-0.43 (0.0041)
Rock Island	16	-0.59 (0.0172)	-	-
Tippecanoe	14	-0.34 (0.2407)	40	-0.57 (0.0001)

DISCUSSION

Ohio Red was shorter than Platte throughout the 1988 and 1989 growing seasons. However, they were not significantly different after June 7, 1989. This slow growth may initially have been related to Ohio Red's slow release from bud dormancy in the greenhouse. Ying and Bagley (1976) suggested that initial growth in *P. deltoides* provenances might be related to early root development. Since Ying and Bagley (1977) had also found that clones from Nebraska had higher numbers of roots per cutting than clones from Ohio, Ohio Red plants might have been at a disadvantage during establishment in 1988. Although Platte was reported by Coleman (1982) to have faster growth than Mighty Mo and Ohio Red, it was not significantly different from Mighty Mo in this study.

There was little apparent relation between the poor growth performance of Rock Island in the provenance plantation at Mead (Table 1) and its water relations or height growth in this study. It showed better height growth than Ohio Red during most of the sample dates. Its growth was slowing, however, by August 1989 when it was the shortest clone due to reduced growth in 1989. The reduced growth might also have been due to damage by mites which was observed in July 1989, mainly on Rock Island saplings. Ying and Bagley (1976) found that some eastern cottonwood provenances had better than average growth at an early age but lost that advantage to other provenances in later years.

In 1988, Ohio Red had lower predawn Ψ_w than Platte (Figure 3a). However, data from 1989 showed no significant difference between the two clones on most of the sample dates. The only exceptions were on May 30, 1989 when Platte had

significantly higher Ψ_w than Ohio Red and on July 11, 1989 when Ohio Red had higher Ψ_w than Platte (Figure 5a). In a study on water relations of Mighty Mo, Ohio Red, and Platte, Coleman (1982) reported that Ohio Red had the highest predawn Ψ_w throughout a 13 day study. Since his plants were in containers and were each supplied with about the same amount of water before the stress treatment, it is possible that Platte's early root development was of little advantage (Ying and Bagley (1977). Coleman (1982) also found that Platte lost less water through transpiration than Ohio Red. The changes observed between 1988 and 1989 in the present study might be due to better establishment of Ohio Red in 1989. The significant difference in average total height between Platte and Ohio Red disappeared in 1989 when all clones were well established. In fact, Ohio Red showed a better growth rate than Platte in 1989.

Osmotic potentials of all clones declined toward the end of the 1988 and 1989 growing seasons due to long dry periods. Similar patterns were reported by Pallardy *et al.* (1983) for three *Quercus* species. Tyree *et al.* (1978) found seasonal and ontogenetic changes in tissue-water relations, including a decline in Ψ_s later in the summer, with several *Populus* species including *Populus deltoides*. Calkin and Pearcy (1984) and Abrams and Knapp (1986) also reported a decline in Ψ_s with season in several woody species. The high predawn Ψ_s values observed on August 2, 1988 (Figure 3b) coincided with a period of generally high growth rates (Figure 2). Predawn Ψ_s declined after August 2, 1988. There was little precipitation during August and none from August 30 to September 13, 1988 (Figure 3d). Predawn Ψ_s

decreased to as low as that of June 28, 1988 when plants were exposed to hot, dry conditions with an average maximum temperature of 37°C for a week.

It is not known whether the seasonal changes observed in predawn Ψ_s were due to solute accumulation (osmotic adjustment) or increased solute concentration due to dehydration. The Ψ_s of rehydrated leaves also followed a similar pattern to predawn Ψ_s measurements, with slightly higher values, both in 1988 and 1989 (Figure 4). This suggests that there was some degree of osmotic adjustment. Sambo and Aston (1985) reported that Ψ_s at full turgor in stressed, rewetted cultivars of *Phalaris tuberosa* (cvv. Australian and Sirosa) was lower than in unstressed control plants. Coleman (1982) found that the average Ψ_s at full turgor for three *P. deltoides* clones was lower under postdrought than predrought conditions, also suggesting osmotic adjustment (Seiler 1985, Sambo and Aston 1985).

Negative correlation coefficients between DWF and Ψ_s were found for all clones studied (Table 3). This type of relationship was also reported by Thomas (1987) in perennial ryegrass (*Lolium perenne* L.). He found that genotypes with the lowest DWF also had the most dilute cell sap and suggested using DWF as a rapid and economical method of screening for Ψ_s in preliminary studies. According to Wilson *et al.* (1980), a change in DWF is one of three processes that account for osmotic adjustment. Variation in the proportion of bound water and accumulation of solutes are the other two processes. In the present study, despite the correlation of DWF to Ψ_s, DWF did not recover from water stress as fast as Ψ_s did. For instance, when plants were under wet conditions and showed high predawn Ψ_w and

Ψ_s on June 27, 1989, DWF values were still as high as they were on June 13, 1989, for all clones. Lower values were observed on the next sample date (July 11, 1989). This slow response of DWF to changing moisture conditions was also observed on diurnal measurements. There was no difference between predawn and afternoon DWF for all clones on a sample date despite a Ψ_s decrease of -0.1 to -0.4 MPa and a Ψ_w decrease of up to -1.1 MPa from predawn to the afternoon (data not shown). Hence, the increase in DWF might be due to substances that are present in stressed leaves that are not removed rapidly by rewatering or partial, overnight recovery from stress. Cutler and Rains (1978) found that drought-hardened cotton plants had a higher DWF than control plants. They suggested that the difference in DWF between hardened and control plants may be accounted for by an increase in soluble solids and insoluble constituents such as cell walls and cuticle in hardened plants. Field-grown *Populus* clones are known to show increased cuticle development when under dry conditions (Pallardy and Kozlowski 1980).

Wilson *et al.* (1980) found stressed leaves of *Macroptilium atropurpureum* had smaller palisade and mesophyll cells, less hemicellulose, more cellulose, and more lignin than unstressed leaves. They suggested that these changes might result in lower water-holding capacity of the walls and might have contributed to the lower bound water content and high DWF observed in stressed leaves. However, Hellkvist *et al.* (1974) found an increase in bound water content in Sitka spruce (*Picea sitchensis* (Bong.) Carr.) shoots in late autumn, which was associated with an increase in DWF. No pressure-volume curves were prepared in the present study and therefore, DWF could not be related to bound water content. Coleman (1982)

however, found lower bound water content after drought than before drought treatments.

Pettersson *et al.* (1957) and Pettersson and Gray (1958) reported that the FWT/DWT ratio (DWF^{-1}) of comparable laminas, which they called "dilation", was inherently higher (lower DWF) for species from wetter habitats than plants from drier habitats. An attempt was therefore made to relate DWF to precipitation of the geographic origins of clones in the present study. Based on normal average annual precipitation (1951 to 1980), Ohio Red comes from the wettest site (104.57 cm), Tippecanoe from an intermediate site (92.96 cm), and Platte from the driest site (73.38 cm)(NOAA 1988). There was no discernible relationship between precipitation and DWF since there were few significant differences in DWF among clones at a given date. When there were significant differences (June 28, August 2, August 30, 1988), Platte had lower DWF values than Tippecanoe. The only exception was on May 30, 1989 when DWF of Platte was greater than Ohio Red and Tippecanoe. Based on the average for all sample dates in 1989, DWF of Platte was significantly higher than that of Tippecanoe but not different from Ohio Red.

In conclusion, all clones showed some degree of acclimation to drought stress, such as reduction in Ψ_s and increase in DWF during dry periods. These are characteristics of drought tolerant plants. Tippecanoe showed the lowest Ψ_s and was generally consistent. Despite low Ψ_s values in 1988, Ohio Red showed higher Ψ_s values than Platte and Tippecanoe. Both Platte and Tippecanoe were the most drought tolerant clones based on Ψ_s. Unlike Ψ_s, which responds to environmental conditions before and during sampling (such as differences in predawn and solar noon samples), DWF was less sensitive to diurnal changes.

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EXPERIMENT II:

SEASONAL AND CLONAL DIFFERENCES IN DEHYDRATION TOLERANCE OF SEVERAL *POPULUS DELTOIDES* CLONES

ABSTRACT

Dehydration tolerance of five field-grown *Populus deltoides* Bartr. clones was investigated during 1988 and 1989 growing seasons by the electrolyte leakage method. An injury index (I_d) was calculated from conductivity changes due to electrolyte leakage during rehydration of dehydrated leaves. Injury index values declined when samples were measured after dry periods for all clones except for a clone from Nebraska ("Platte"). Platte showed consistently low I_d values in 1988. In 1989, when I_d values increased following favorable weather conditions (June and July), Platte and a clone from Indiana (Tippecanoe) had lower I_d values than a clone from Ohio (Ohio Red). There were no significant differences in I_d among clones when plants were sampled after a dry period, indicating that all clones had drought-hardened during the period.

INTRODUCTION

The ability of plants to endure water deficits at low tissue water potentials is known as drought tolerance (Levitt 1980, Kramer 1983). Levitt (1980) subdivided drought tolerant plants into two groups depending on the mechanism of tolerance: 1) dehydration avoiders, those that maintain cell turgor by osmotic adjustment, and 2) dehydration tolerators, those that show protoplasmic tolerance. Dehydration tolerant

plants either show less damage or show better recovery from damage due to water stress than less tolerant plants. Giles *et al.* (1976) reported that complete tonoplast breakdown of maize (*Zea mays* L.) leaves occurred at a leaf water potential of -1.8 MPa while sorghum (*Sorghum bicolor* L.) leaves showed partial tonoplast breakdown at -3.7 MPa.

One method of selecting dehydration tolerant plants is based on the ability of the cell membrane to control solute leakage (Sullivan and Eastin 1974). Solute or electrolyte leakage from a sample is obtained by measuring either UV-absorbing substances or measuring the conductivity of electrolytes leaked into solution. An index of injury is then calculated to compare species or genotypes. This method has been used for freezing (Flint *et al.* 1967) and dehydration tolerance studies of annual crops (Sullivan and Ross 1974, Shcherbakova and Kacperska 1983) and woody plants (Martin *et al.* 1987). Most of these studies have been on leakage differences between species, but this method has also been used to compare cultivars of some crop plants (Sullivan and Ross 1979, Blum and Ebercon 1981).

Genetic variability in drought tolerance among *Populus* clones has been reported by several authors (Kelliher and Tauer 1980, Pallardy and Kozlowski 1981). In a study with eastern cottonwood (*P. deltoides* Bartr.), Kelliher and Tauer (1980) found plants from a dry-site had lower stomatal resistances than plants from a wet-site under both wet or dry conditions. Their dry-site plants showed a characteristic of drought tolerance in keeping their stomata open at low water potentials (Levitt 1980).

In this study the existence and extent of dehydration tolerance in five *Populus deltoides* clones was investigated by the electrolyte leakage method. Seasonal variations in dehydration tolerance were also investigated.

MATERIALS AND METHODS

Plant Material

The plant material used for this experiment is described in experiment 1. Briefly, forty cuttings each of five clones originally from Illinois ("Rock Island"), Indiana ("Tippecanoe"), Missouri ("Mighty Mo"), Nebraska ("Platte"), and Ohio ("Ohio Red") were planted at the University of Nebraska-Lincoln (UNL) greenhouse on March 16, 1988. They were transplanted to a field plot on the UNL campus on May 12, 1988.

Leaf Sampling and Water Status Measurements

Three sampling dates were used in 1988 and four in 1989. In 1988, five plants per clone were randomly selected on each of three dates; July 19, August 16, and September 13. Five fully expanded leaves from each of the five selected plants were then detached and the petiole was immediately placed in a labelled test tube filled with water. These leaves were brought to the laboratory, placed in the dark with a plastic cover, and allowed to rehydrate for 24 hours. This brought leaf water potential (Ψ_w) to approximately -0.1 MPa.

Twenty-five leaves (1 leaf per plant, 5 plants per clone, 5 clones) were randomly assigned to a no-stress treatment after rehydration. Four, 1-cm discs were

sampled from each leaf with a cork borer and placed in a test tube. The midrib and main veins of leaves were avoided in all samples. The Ψ_w of each leaf was then measured with a pressure chamber (Ritchie and Hinckley 1975, Turner 1981). In a preliminary study, removal of three or four 1-cm discs from eastern cottonwood leaves did not affect Ψ_w readings. This finding has been reported for several other species by Martin *et al.* (1987).

The leaf discs were rinsed with two changes of distilled, deionized water (Sullivan and Ross 1979). After rinsing, 7 ml of distilled, deionized water was added to each tube. The tubes were then placed in an incubator at 10°C for 22 to 24 hours (Sullivan and Ross 1979, Blum and Ebercon 1981). This method was preferred over shaking samples at room temperature as described by Martin *et al.* (1987), in order to minimize secondary effects of shaking and metabolic activity (Sullivan and Ross 1979). Shcherbakova and Kacperska-Palacz (1980) also found that holding at room temperature promoted tissue damage in immersed tissue while storage at 5°C did not.

The remaining rehydrated leaves were randomly assigned to one of four stress levels (25 leaves to each level). These leaves were placed in perforated plastic bags and allowed to dry on a bench in a dark room at room temperature. Four levels of perforation were used in order to accomplish different degrees of stress and complete the sampling within 24 hours. Periodically during drying, leaf discs were sampled from all leaves assigned to a certain stress level. The procedures for disc sampling, Ψ_w measurements, and incubator storage for the stressed leaves were the same as for the nonstressed leaves.

Relative water content (RWC) was measured for all treatments in July 1988 on 15 randomly selected leaves (1 leaf per plant, 3 plants per clone, 5 clones) after Ψ_w readings were taken. Relative water content was measured on all leaves sampled (25 leaves per treatment) in August and September 1988. Relative water content measurement procedures are described in experiment 1.

Electrolyte Leakage Measurement

The solution conductivity of each tube with discs was read with a conductance meter (YSI Model 35 and YSI 3403 cell, Yellow Springs Scientific Instruments Co., Yellow Springs, OH) at 25°C after shaking for one hour at a moderate speed (180 cycles min^{-1}) on an orbital shaker. Samples were then autoclaved for 15 min to completely lyse cell membranes, cooled to room temperature, shaken for 10 min, and a second conductivity reading taken at 25°C (Sullivan 1972, Blum and Ebercon 1981, Martin *et al.* 1987).

Electrolyte leakage was quantified by computing an injury index (I_d) as described by Flint *et al.* (1967) for studies of freezing injury. This formula has also been used for dehydration studies (Dlugokecka and Kacperska-Palacz 1978, Martin *et al.* 1987). It is calculated by:

$$I_d = 100\ (R_d - R_o)/(1 - R_o)$$

where:

I_d = injury index in percent;

R_d = ratio of solution conductivity from discs of stressed leaves before autoclaving, to conductivity of the same sample after autoclaving.

R_o = ratio of solution conductivity from discs of nonstressed leaves before autoclaving, to conductivity of the same sample after autoclaving.

An I_d of zero means no leakage and an I_d of 100 means complete lysing of cell membranes and release of cell contents.

An initial plan to compare changes in I_d with changes in Ψ_w was abandoned because higher Ψ_w readings were sometimes obtained for severely stressed leaves (RWC < 50%) than for less stressed leaves. This might have been due to the effect of matric potential, which is normally ignored under moderate water stress (Hsiao 1973). Preliminary analysis of July data and data from an unidentified cottonwood coppice near the study plot showed a sigmoid relationship between RWC and I_d, similar to that shown for heat stress in sorghum by Sullivan (1972).

While Ψ_w readings of all sampled leaves (25 leaves -- 5 per clone) were taken in July, only 15 leaves (3 per clone) were used for RWC on that date. On the remaining sample dates, RWC of all sampled leaves was measured and one more group of 25 leaves was included which increased the number of stress levels from four to five.

The 1989 sample dates were May 30, June 27, July 28, and August 21. The number of plants sampled per clone was increased to seven and the total number of leaves collected from each clone was 42 (6 per plant). Selection of a plant was at random amongst all plants with sufficient leaves. Some plants were sampled on more than one date because of the limited number of individuals available in some clones. Water relations and leakage measurements were done as in 1988.

Data Analysis

Relative water content could not be precisely controlled due to variability in the rate of desiccation among and between clones. Predictive equations were therefore developed between I_d (dependent variable) and powers of RWC (independent variable), with a stepwise regression technique (Martin *et al.* 1987). For each clone and sampling date, a model containing all significant variables ($\alpha \leq 0.05$) was used to generate values of I_d within the range of RWC measured. Using the mean square error of each predictive equation, the standard error of a predicted I_d at a given RWC was calculated from a generalized inverse matrix. The standard error was then used to calculate the 95% confidence interval of the predicted I_d at a given RWC (Draper and Smith 1981). The confidence intervals were used to compare clones at chosen RWC values (Appendix B). A water stress level of 60% RWC was selected for comparison purposes. Since no actual RWC was measured for severely stressed plants in the field, this was assumed to be the lowest RWC value these clones would survive. Following record high temperatures (up to 41°C) and a dry period in June 1988, plants were measured that survived a predawn Ψ_w of -2.3 MPa. Scarascia-Mugnozza *et al.* (1986) reported zero leaf conductance below a Ψ_w of -2.5 MPa for *P. deltoides* plants. The corresponding Ψ_w values for a RWC of 60% ranged from -2.5 to -3.3 MPa. Since clones had different Ψ_w values at the same RWC, a lower RWC value of 55% was also included. The corresponding Ψ_w values for a RWC of 60% ranged from -2.5 to -3.3 MPa and for 55% RWC, the range was from -2.8 to -3.5 MPa.

RESULTS

1988 Growing Season

Figures 1 and 2 show the relationship between I_d and RWC among clones and sampling dates, respectively, predicted from regression equations. Figure 1 also shows the general pattern of the relationship between I_d and RWC observed for all sampling dates. There were no significant differences among clones in I_d for RWC at or above 65%. Below a RWC of 45%, predicted I_d values were less reliable due to fewer observations. The highest I_d values of the season were observed on July 19 for all clones except Platte (Table 1). All clones except Platte also showed a sharp decline in I_d on August 16. Injury indexes of Rock Island and Tippecanoe increased slightly on September 13. At a RWC of 60%, only Platte maintained the same low I_d (3.2 to 3.4%) throughout the season, although there was a slight increase below a RWC of 60% in August (Figure 2). Ohio Red showed the highest rate of leakage as RWC declined during the season except on August 1988 (Figure 1).

Table 1. Injury index (I_d) values at 60% and 55% relative water content (RWC) for 1988 sample dates. At a given RWC and date, I_d values with the same letters are not significantly different ($\alpha \leq 0.05$).

	July 19		August 16		September 13	
Clone	60%	55%	60%	55%	60%	55%
Mighty Mo	22.40ab	33.81a	2.41a	12.51a	3.79ab	11.76a
Rock Island	20.62ab	33.12a	3.27a	5.75ab	7.38a	13.80a
Ohio Red	19.02a	39.91a	5.47a	12.21a	5.23ab	16.00a
Tippecanoe	10.29bc	20.99b	1.26a	2.99b	7.18ab	14.85a
Platte	3.21c	10.09c	3.41a	13.63a	3.37b	5.11b

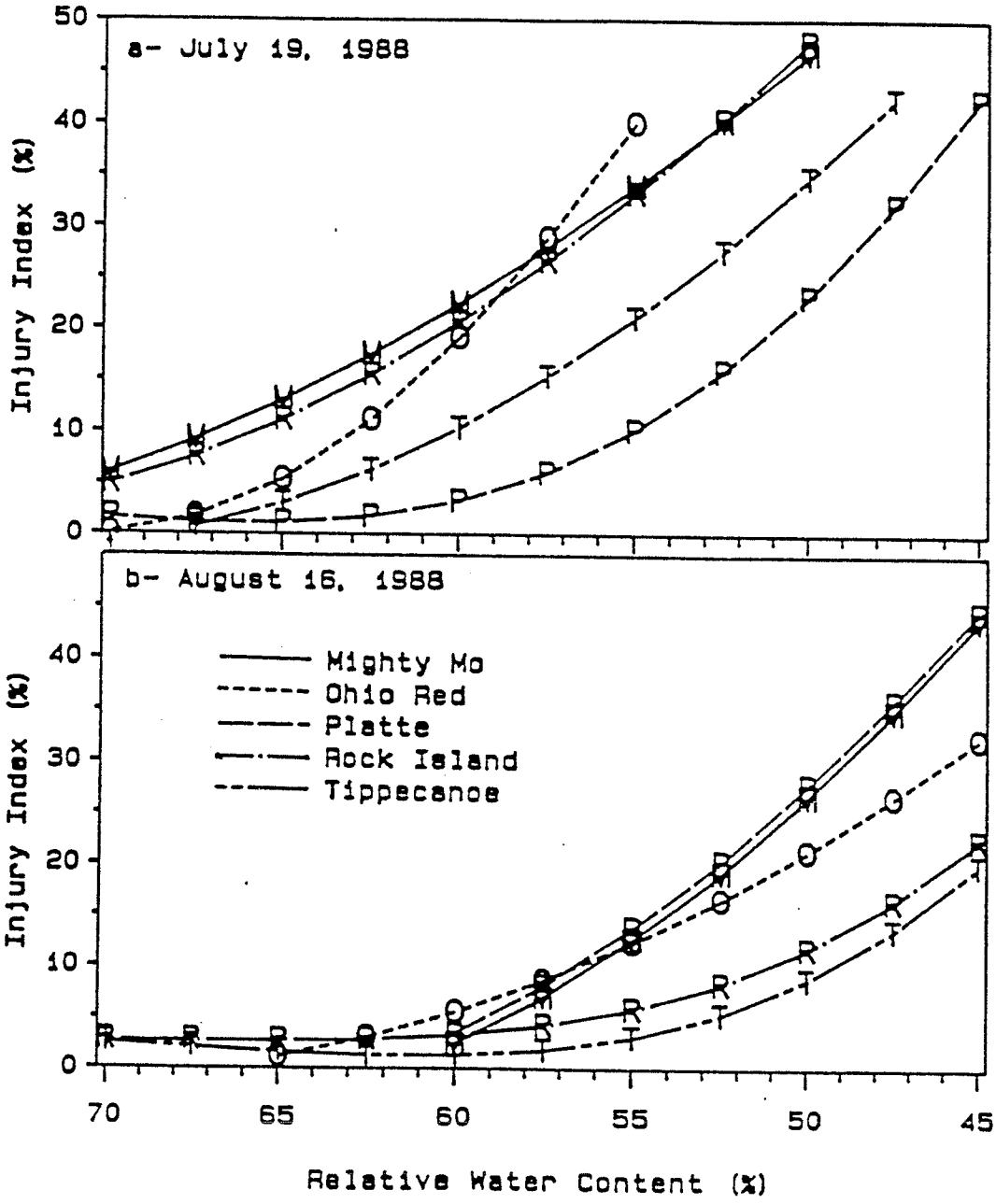
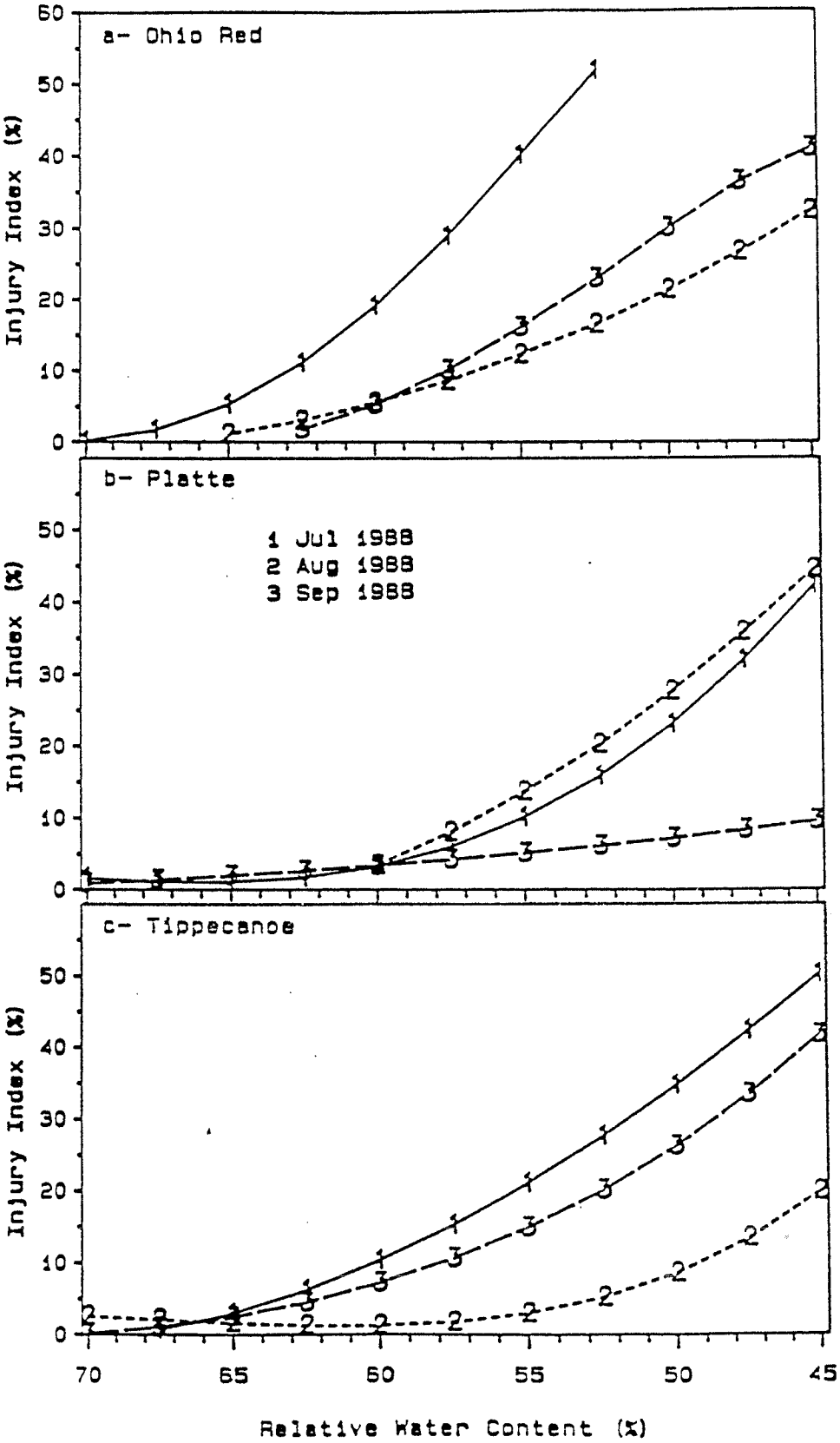


Figure 1. Relationship between injury index and relative water content of five *Populus deltoides* clones predicted by regression equations for (a) July 19, and (b) August 16, 1988.

Figure 2. Seasonal variation in injury index versus relative water content for *Populus deltoides* clones, (a) Ohio Red, (b) Platte, and (c) Tippecanoe in 1988.


a- Ohio Red
b- Platte
c- Tippecanoe
1 Jul 1988
2 Aug 1988
3 Sep 1988
Injury Index (%)
Relative Water Content (%)
0
10
20
30
40
50
60
70
65
55
45

At a RWC of 60%, Platte showed significantly lower I_d than Mighty Mo, Ohio Red, and Rock Island on July 19 (Table 1). Platte's I_d was also significantly lower (3.37%) than Rock Island's (7.38%) in September. Tippecanoe had significantly lower I_d than Ohio Red in July. The remaining clones were not significantly different from each other throughout the season. In the August samples, no differences were detected among clones at RWC 60% although Rock Island and Tippecanoe had lower values than Platte, Mighty Mo, and Ohio Red at lower RWC. Below a RWC of 60%, I_d values for Platte decreased in September from that of August (Figure 2). No significant difference was observed in I_d for Mighty Mo and Ohio Red between August and September at a RWC of 55%.

1989 Growing Season

The lowest I_d values of the season (4.6 to 7.6%) were observed in May for all clones studied (Table 2). Clonal and seasonal variations in I_d are shown on Figures 3 and 4 respectively. Figure 3 represents the pattern of the relationship between I_d and RWC observed during the season. No significant differences were found among clones at a RWC of 65% or higher except in June when Tippecanoe

Table 2. Injury index (I_d) values at 60% and 55% relative water content (RWC) for 1989 sample dates. For a given RWC and date, I_d values with the same letters are not significantly different ($\alpha \leq 0.05$).

	May 30		June 27		July 28		August 21	
Clone	60%	55%	60%	55%	60%	55%	60%	55%
Ohio Red	5.75a	9.98a	26.09a	42.66a	22.98a	36.85a	14.99a	26.49a
Platte	4.64a	7.76a	13.55b	18.79b	13.49b	25.38b	14.83a	26.47a
Tippecanoe	7.61a	11.08a	9.95b	19.26b	12.86b	20.96b	11.21a	18.64a

had significantly lower I_d (3.65%) than Platte (9.11%) and Ohio Red (13.9%). Injury index values increased in June from that of May by 354%, 192%, and 31% for Ohio Red, Platte, and Tippecanoe, respectively, at 60% RWC (Figure 4). After the peak in June, only Ohio Red showed decline in leakage throughout the remaining sample dates (Table 2). At a RWC of 60%, I_d values of Platte and Tippecanoe remained at about the same level as they were in June for the remaining sampling dates and were significantly lower than Ohio Red both in June and July. There were no significant differences among clones in May and August. Average predawn Ψ_w reached -0.7 MPa in August as shown in experiment 1, Figure 5. Plants received a total of only 7.6 and 13.2 mm of precipitation in three weeks before May 30 and August 21 respectively, while the total precipitation received in a three week period before June sampling was 138.2 mm, most of it (98.6 mm) within a week before sampling (Table 3).

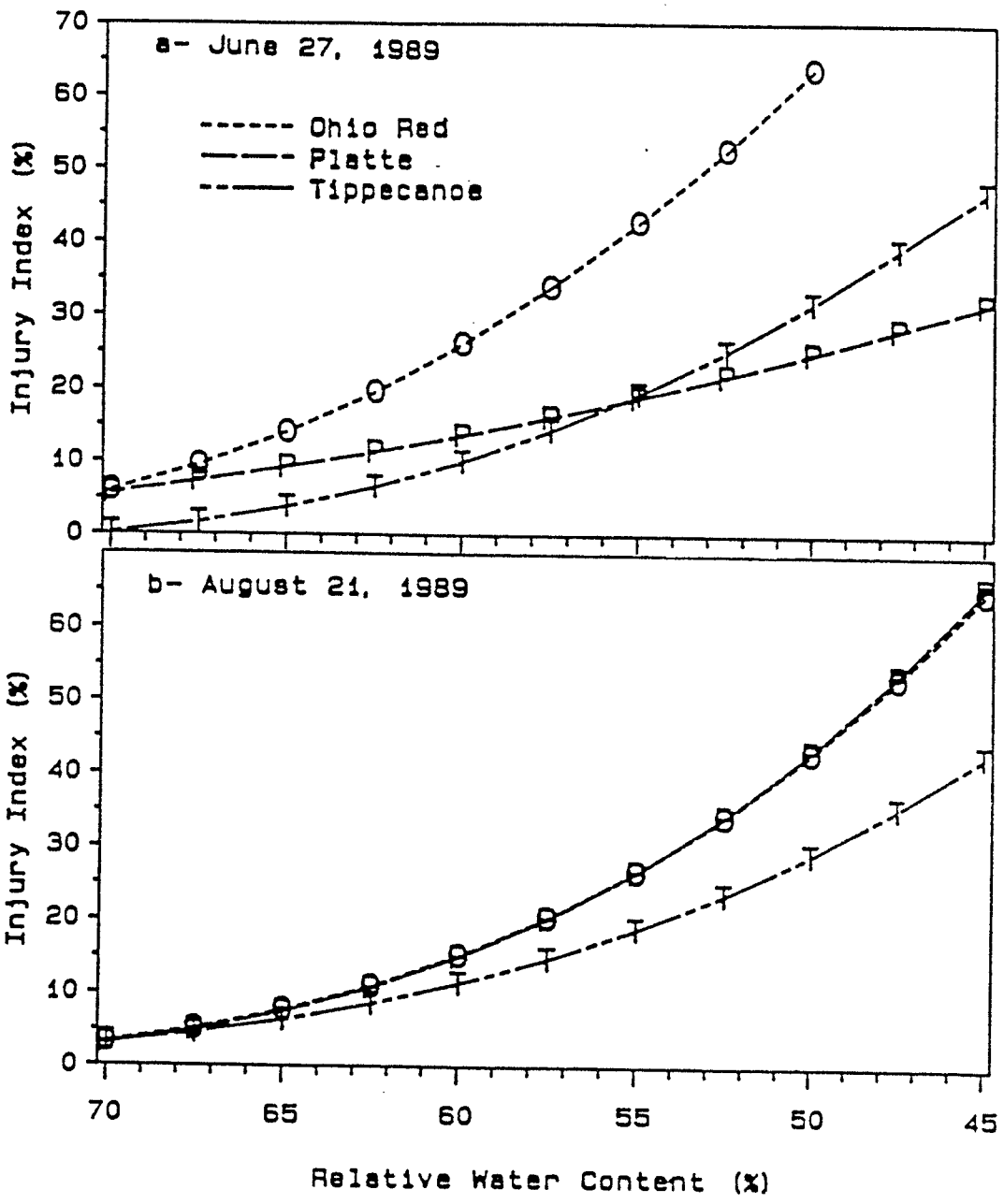


Figure 3. Relationship between injury index and relative water content of three *Populus deltoides* clones predicted by regression equations for (a) June 27, and (b) August 21, 1989.

Figure 4. Seasonal variation in injury index versus relative water content for *Populus deltoides* clones, (a) Ohio Red, (b) Platte, and (c) Tippecanoe in 1989.

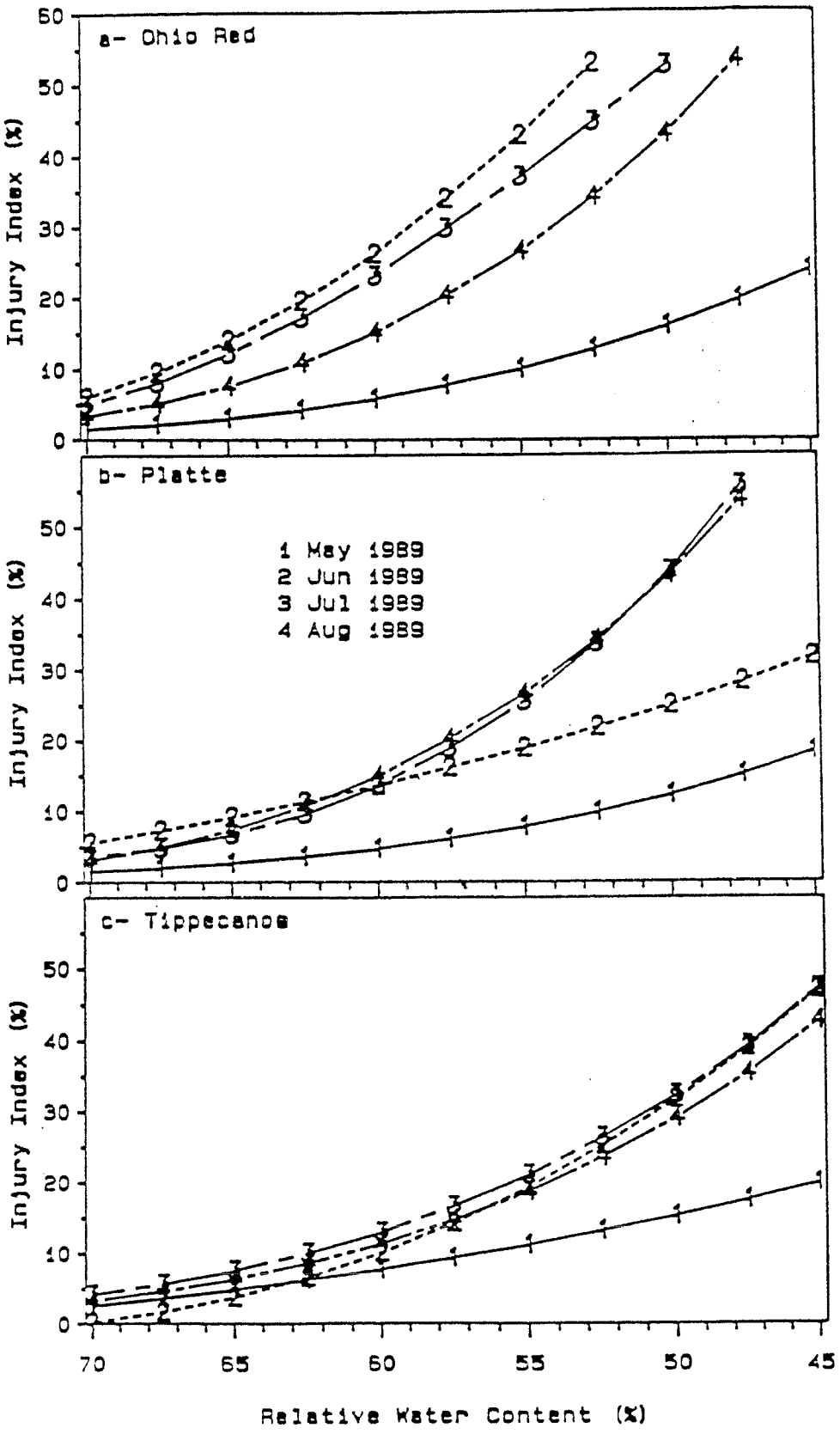

a- Ohio Red
b- Platte
c- Tippecanoe
1 May 1989
2 Jun 1989
3 Jul 1989
4 Aug 1989
Injury Index (%)
Relative Water Content (%)
0
10
20
30
40
50
60
70
65
60
55
50
45

Table 3. Weekly average maximum temperature (°C) and total precipitation (mm)[1] three weeks before each sampling date in 1988 and 1989 (irrigation shown in parenthesis).

Sampling Date	Date	Average maximum Temperature (°C)	Total Precipitation (mm)
1988			
	6/28-7/04	28.10	4.32
7/19	7/05-7/11	32.14	53.59 (45.50)
	7/12-7/18	34.60	50.60 (50.60)
	7/26-8/01	34.13	23.60 (23.10)
8/16	8/02-8/08	33.81	36.32 (14.00)
	8/09-8/15	35.16	40.89 (10.90)
	8/23-8/29	28.81	1.78
9/13	8/30-9/05	28.10	0.00
	9/06-9/12	29.92	0.00
1989			
	5/09-5/15	24.37	0.00
5/30	5/16-5/22	26.35	5.08
	5/23-5/29	28.57	2.54
	6/06-6/12	27.06	34.04
6/27	6/13-6/19	26.75	5.59
	6/20-6/26	29.21	98.55
	7/07-7/13	35.24	13.46
7/28	7/14-7/20	26.03	39.62
	7/21-7/27	29.92	2.54
	7/31-8/06	31.83	3.05
8/21	8/07-8/13	31.03	0.00
	8/14-8/20	30.95	10.16

[1]Weather data obtained from Center for Agricultural Meteorology and Climatology (CAMaC), UNL.

DISCUSSION

These results show that *P. deltoides* clones differ in their ability to control electrolyte leakage when dehydrated. There was also seasonal variation in electrolyte leakage and in the pattern of differences between the clones. Leakage of electrolytes has been used as a measure of cell membrane damage due to dehydration by many investigators (Sullivan and Eastin 1974, Blum and Ebercon 1981, Shcherbakova and Kacperska 1983). Based on lower I_d values at 60% RWC, Platte and Tippecanoe were more dehydration tolerant than Ohio Red. There was some relationship between dehydration tolerance and clonal origin because Platte came from a drier site than Ohio Red. Based on the average from 1951 to 1980, the original collection sites of the clones had annual precipitation of 73.38, 92.96, and 104.57 cm for Platte, Tippecanoe, and Ohio Red, respectively, although May to September precipitation was about the same (47 to 50 cm) for all sites (NOAA 1988). Rock Island was not different from Mighty Mo and Ohio Red in I_d for all sample dates. The poor growth performance of Rock Island at Mead (see Table 1 in experiment 1) could not be related to its dehydration tolerance in the first growing season.

During the 1989 growing season, plants were exposed to more water stress in May than in June, and I_d values were lower in May, probably due to a longer period of drought hardening. Predehydrated plants have been reported to show less leakage than non-predehydrated plants. Shcherbakova and Kacperska-Palacz (1980) reported that electrolyte leakage from desiccated rape hypocotyls (*Brassica napus* L. var. *oleifera* L. cv. Gorczanski) was lower in hardened (predehydrated to about 40%

RWC) than non-hardened tissues. Increased dehydration tolerance after drought hardening was also reported in soybean (*Glycine max* L. Merr.)(Krishnamani *et al.* 1984) and cabbage (*B. oleracea* var. *capitata*, Early Jersey Wakefield)(Levitt 1985).

Plants received sufficient precipitation in June 1989 (Table 3) and leakage was greater for all clones in June than in May (Figure 4). The increase was higher for Ohio Red (354%) than for Platte (192%) or Tippecanoe (31%) at a RWC of 60%. Such an increase was an indication that drought hardening against membrane damage in these clones has limits similar to those reported for osmotic adjustment in other plants. Turner and Jones (1980) reported that osmotic adjustment, which improves when plants are drought hardened, was lost a few days after plants recovered fully from drought stress, and there was no advantage for subsequent drought cycles. It is possible that there is a relationship between hardening that results in osmotic adjustment and hardening that results in reduced leakage. Blum and Ebercon (1981) suggested a phenomenon of adjustment in cell membrane stability for plants that show decreased electrolyte leakage with predehydration treatment, similar to those that adjust osmotically when predehydrated.

Although leakage of Ohio Red continued declining from June 1989 on, none of the clones reached the low I_d obtained in May 1989. In fact, Platte showed a 35% increase from June to August at 55% RWC (Figure 4b). Martin *et al.* (1987) observed drought hardening in several woody species exposed to high temperature and low precipitation. They found reduced leakage for three *Quercus* species and *Cornus florida* L. late in the season, while leakage increased or remained the same for species from more mesic sites (*Juglans nigra* L. and *Acer saccharum* Marsh.).

It is possible that Platte requires a longer exposure period under dry conditions before it can show hardening. This also might be the reason for the increased I_d in August and decline in September 1988 when plants were sampled after a two week dry period. Rate of stress development, degree of stress, light, and temperature conditions are reported to affect osmotic adjustment of several plants (Turner and Jones 1980). Membrane adjustment may also be affected by similar factors.

Except in May 1989, I_d values for clones that were included in both years were higher in 1989 than in 1988, probably due to higher precipitation and cooler temperatures in 1989 (Table 3). This is in agreement with Blum and Ebercon (1981) who reported that leakage during a year with favorable water regime was higher than a year with prolonged water stress period in several wheat (*Triticum* spp.) cultivars. They also reported that maximum cultivar separation in I_d occurred when the plants were under favorable moisture. In the present study, June and July, 1989 were months with the best precipitation for the season. It was also during these months that significant differences were found among clones, when Platte and Tippecanoe had significantly lower I_d than Ohio Red. Samples taken during the dry months showed no significant differences in I_d among clones. Ohio Red showed reduced leakage when exposed to more dry weather (Figures 2 and 4). This indicates that Ohio Red responds to hardening and can be as tolerant as Platte and Tippecanoe when predehydrated.

Clonal and seasonal variations in I_d were found. All clones responded to drought hardening by reducing leakage. Platte and Tippecanoe showed better dehydration tolerance than Ohio Red, Mighty Mo, and Rock Island.

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EXPERIMENT CONCLUSIONS

Since Platte had been reported to show higher leaf resistance than Ohio Red and Mighty Mo, it was expected to show a drought avoidance response. High predawn leaf water and osmotic potentials observed for Platte in 1988 seemed to support this. However, 1989 results were different, with Platte having lower predawn leaf water and osmotic potentials than Ohio Red. Platte also showed lower electrolyte leakage than both Ohio Red and Mighty Mo. In fact, all clones showed some degree of hardening, a property of drought tolerance, as evidenced by the lower osmotic potentials, increases in dry weight fractions, and reduction in electrolyte leakages that occurred when plants were exposed to dry periods before sampling. Osmotic potentials of Tippecanoe and Ohio Red were mostly lower than for Platte in 1988. In the second year, Ohio Red showed mostly higher osmotic potentials than Platte and Tippecanoe. The reason for higher osmotic potentials for Platte than Ohio Red in 1988 and the reverse in 1989 is not clear but it might be due to difference in establishment related to root development.

Platte and Tippecanoe showed better dehydration tolerance than Ohio Red, Mighty Mo, and Rock Island by showing less electrolyte leakage on rehydration. These clones should be selected for further study of membrane components such as proteins and/or phospholipids, and of other factors such as osmotic adjustment.

During the 1989 sampling dates when both electrolyte leakage and predawn leaf osmotic potentials were measured (June 27, July 28, and August 21), Platte and Tippecanoe had significantly lower osmotic potentials than Ohio Red. Platte and Tippecanoe also had significantly lower electrolyte leakage than Ohio Red on the

first two dates. On August 21, 1989 the osmotic potentials of Platte and Ohio Red were not significantly different, but Ohio Red had significantly higher osmotic potential than Tippecanoe. Although there were no significant differences among clones on this date in electrolyte leakage, Ohio Red showed a higher value than Tippecanoe. This relationship between low osmotic potential and low electrolyte leakage, if it can be repeated in other experiments, indicates the need to study the role of solutes that are involved in osmotic adjustment in relation to membrane injury. Hoekstra *et al.* (1989) had reported that the presence of sucrose in dry pollen of corn and *Pennisetum typhoides* was a key factor in preserving membranes.

The electrolyte leakage method was successful in detecting differences in dehydration tolerance among clones of *P. deltoides*. Leakage measurements for samples that received mild stress (RWC above 80%) were sometimes negative, probably related to error in measuring conductivities lower than the recommended limit for accuracy of the instrument. Increasing the number of leaf discs sampled might reduce such errors. Using relative water content as an independent variable can be advantageous in that more than four discs can be sampled from each leaf for leakage. The number of leaf discs sampled was limited in order to minimize the effect of disc sampling on water potential readings.

Only detached leaves were dehydrated during the study on electrolyte leakage. Further study is recommended on discs sampled directly from leaves attached to plants that are water stressed to different levels under controlled environmental conditions. Since there would be variability in electrolyte leakage among plants within a clone, a method should be developed to overcome the

problem of getting leaves that would serve as controls. A leaf from each plant may be detached and rehydrated as a control. However, solutes from severely stressed plants are likely to leak out during the rehydration process. Also one should anticipate the effect of diseases such as canker which might appear (as it did in the field study in 1988) during severe dry periods.

Further study is also recommended on the relationship between bound water and dry weight fraction. The same leaf can be used for osmotic potential and dry weight fraction measurements.

APPENDIX A: ANALYSIS OF VARIANCE AND MEANS OF LEAF WATER POTENTIAL, OSMOTIC POTENTIAL, DRY WEIGHT FRACTION, AND HEIGHT DURING 1988 AND 1989 GROWING SEASONS

Table A.1. Analysis of variance for 1988 measurements of predawn leaf water potential and osmotic potential.

Sample Date	Source of Variation	Degrees of Freedom	Sums of Squares	F Value	P > F
		Water potential			
6/14/88	Clone	4	0.0984	2.69	0.0784
	Error	13	0.1190		
6/28/88	Clone	4	0.6065	1.66	0.2295
	Error	11	1.0071		
7/19/88	Clone	4	0.0486	0.70	0.6099
	Error	10	0.1738		
8/02/88	Clone	4	0.2827	4.58	0.0232
	Error	10	0.1542		
8/16/88	Clone	4	0.2354	6.17	0.0091
	Error	10	0.0954		
8/30/88	Clone	4	0.2681	3.65	0.0441
	Error	10	0.1838		
9/13/88	Clone	4	0.2588	4.48	0.0289
	Error	10	0.1300		
9/27/88	Clone	4	0.1700	4.81	0.0201
	Error	10	0.0883		
		Osmotic potential			
6/14/88	Clone	4	0.0556	1.69	0.2129
	Error	13	0.1072		
6/28/88	Clone	4	0.0622	5.54	0.0129
	Error	10	0.0281		
7/19/88	Clone	4	0.0454	0.08	0.9861
	Error	10	1.3833		
8/02/88	Clone	4	0.2486	3.33	0.0560
	Error	10	0.1868		
8/16/88	Clone	4	0.1169	6.05	0.0097
	Error	10	0.0483		
8/30/88	Clone	4	0.1173	6.28	0.0086
	Error	10	0.0467		
9/13/88	Clone	4	0.0589	0.87	0.5160
	Error	10	0.1699		
9/27/88	Clone	4	0.1019	1.72	0.2226
	Error	10	0.1485		

Table A.2. Analysis of variance for 1989 measurements of predawn leaf water potential and osmotic potential.

Sample Date	Source of Variation	Degrees of Freedom	Sums of Squares	F Value	P > F
			Water potential		
5/30/89	Clone	2	0.0554	12.64	0.0004
	Error	18	0.0395		
6/13/89	Clone	2	0.0034	2.31	0.1279
	Error	18	0.0132		
6/27/89	Clone	2	0.0004	2.33	0.1256
	Error	18	0.0016		
7/11/89	Clone	2	0.0954	5.08	0.0178
	Error	18	0.1689		
7/28/89	Clone	2	0.0371	3.06	0.0719
	Error	18	0.1093		
8/09/89	Clone	2	0.0016	0.02	0.9798
	Error	18	0.7071		
8/21/89	Clone	2	0.0859	2.45	0.1145
	Error	18	0.3155		
			Osmotic potential		
6/13/89	Clone	2	0.0571	10.25	0.0011
	Error	18	0.0501		
6/27/89	Clone	2	0.2182	12.51	0.0005
	Error	17	0.1483		
7/11/89	Clone	2	0.1448	13.49	0.0003
	Error	18	0.0966		
7/28/89	Clone	2	0.0678	6.41	0.0079
	Error	18	0.0953		
8/09/89	Clone	2	0.0017	0.11	0.9003
	Error	18	0.1414		
8/21/89	Clone	2	0.0347	3.97	0.0372
	Error	18	0.0787		

Table A.3. Analysis of variance for 1988 and 1989 measurements of dry weight fraction.

Sample Date	Source of Variation	Degrees of Freedom	Sums of Squares	F Value	P > F
1988					
6/14	Clone	4	0.00024	0.30	0.8722
	Error	13	0.00263		
6/28	Clone	4	0.00238	2.09	0.1504
	Error	11	0.00314		
7/19	Clone	4	0.00490	0.75	0.5775
	Error	10	0.01625		
8/02	Clone	4	0.00210	2.12	0.1534
	Error	10	0.00248		
8/16	Clone	4	0.00054	1.17	0.3794
	Error	10	0.00116		
8/30	Clone	4	0.00069	3.65	0.0439
	Error	10	0.00048		
9/13	Clone	4	0.00066	6.83	0.0082
	Error	10	0.00022		
9/27	Clone	4	0.00107	1.14	0.3937
	Error	10	0.00236		
1989					
5/30	Clone	2	0.00226	9.99	0.0012
	Error	18	0.00204		
6/13	Clone	2	0.00017	2.60	0.1017
	Error	18	0.00057		
6/27	Clone	2	0.00051	1.46	0.2607
	Error	17	0.00295		
7/11	Clone	2	0.00041	1.12	0.3479
	Error	18	0.00333		
7/28	Clone	2	0.00038	2.27	0.1324
	Error	18	0.00151		
8/09	Clone	2	0.00001	0.03	0.9686
	Error	18	0.00325		
8/21	Clone	2	0.00051	2.01	0.1636
	Error	18	0.00231		

Table A.4. Analysis of variance for 1988 measurements of water potential, osmotic potential, and dry weight fraction at solar noon (1 P.M. Central Daylight Time).

Sample Date	Source of Variation	Degrees of Freedom	Sums of Squares	F Value	P > F
			Water potential		
6/14/88	Clone	4	0.0913	6.78	0.0036
	Error	13	0.0438		
6/28/88	Clone	4	0.3683	6.70	0.0069
	Error	10	0.1375		
			Osmotic potential		
6/14/88	Clone	4	0.1627	3.94	0.0262
	Error	13	0.1342		
6/28/88	Clone	4	0.0567	1.63	0.2422
	Error	10	0.0870		
			Dry weight fraction		
6/14/88	Clone	4	0.00068	1.19	0.3598
	Error	13	0.00187		
6/28/88	Clone	4	0.00094	0.87	0.5141
	Error	10	0.00270		

Table 5. Analysis of variance for 1988 and 1989 measurements of height growth.

Sample Date	Source of Variation	Degrees of Freedom	Sums of Squares	F Value	P > F
1988					
5/07	Clone	4	2485.40	10.67	0.0001
	Error	39	2270.76		
6/07	Clone	4	3353.87	13.94	0.0001
	Error	40	2406.44		
7/11	Clone	4	4939.68	9.68	0.0001
	Error	40	5101.61		
8/10	Clone	4	7799.05	4.98	0.0024
	Error	40	15660.90		
9/19	Clone	4	12069.01	3.41	0.0172
	Error	40	35388.82		
1989					
5/11	Clone	4	12498.04	2.96	0.0312
	Error	40	42245.08		
6/11	Clone	4	14528.14	2.02	0.1107
	Error	40	72091.97		
7/12	Clone	4	7896.76	0.60	0.6627
	Error	40	130958.52		
8/09	Clone	4	8892.04	0.56	0.6939
	Error	40	159175.26		

Table A.6. Mean leaf water potential, osmotic potential (MPa), and dry weight fraction during 1988 growing season. Standard errors of mean are shown in parentheses (number of leaves = 3).

Clone	Jun 14	Jun 21	Jun 28	Jul 19	Aug 2	Aug 16	Aug 30	Sep 13	Sep 27
				Mean predawn water potential (MPa)					
Mighty Mo	-0.26	-0.39	-0.39	-0.08	-0.28	-0.25	-0.18	-0.25	-0.08
	(0.05)	(0.03)	(0.05)	(0.03)	(0.02)	(0.03)	(0.02)	(0.08)	(0.03)
Ohio Red	-0.43	-0.42	-0.90	-0.14	-0.48	-0.33	-0.44	-0.55	-0.33
	(0.04)	(0.04)	(0.22)	(0.03)	(0.04)	(0.04)	(0.06)	(0.05)	(0.12)
Platte	-0.31	-0.30	-0.48	-0.08	-0.17	-0.30	-0.19	-0.16	-0.05
	(0.08)	(0.02)	(0.19)	(0.01)	(0.04)	(0.05)	(0.03)	(0.02)	(0.00)
Rock Island	-0.34	-0.54	-0.44	-0.23	-0.36	-0.56	-0.43	-0.21	-0.15
	(0.03)	(0.05)	(0.03)	(0.16)	(0.09)	(0.05)	(0.05)	(0.03)	(0.00)
Tippecanoe	-0.47	-0.45	-0.58	-0.08	-0.55	-0.53	-0.50	-0.42	-0.05
	(0.02)	(0.02)	(0.17)	(0.02)	(0.12)	(0.10)	(0.15)	(0.12)	(0.00)
				Mean predawn osmotic potential (MPa)					
Mighty Mo	-1.80	-1.87	-2.00	-2.00	-1.59	-1.62	-1.83	-1.97	-2.08
	(0.07)	(0.02)	(0.03)	(0.21)	(0.07)	(0.02)	(0.03)	(0.08)	(0.14)
Ohio Red	-1.74	-1.77	-2.09	-1.91	-1.63	-1.68	-1.79	-2.08	-1.87
	(0.02)	(0.02)	(0.02)	(0.15)	(0.08)	(0.04)	(0.02)	(0.10)	(0.03)
Platte	-1.79	-1.71	-1.98	-1.83	-1.40	-1.56	-1.73	-1.89	-1.86
	(0.05)	(0.08)	(0.04)	(0.25)	(0.05)	(0.05)	(0.03)	(0.02)	(0.03)
Rock Island	-1.72	-1.88	-2.01	-1.90	-1.59	-1.73	-1.82	-1.96	-2.00
	(0.05)	(0.06)	(0.03)	(0.2)	(0.09)	(0.02)	(0.05)	(0.03)	(0.07)
Tippecanoe	-1.89	-1.88	-2.15	-1.93	-1.80	-1.81	-2.00	-2.02	-1.99
	(0.01)	(0.02)	(0.03)	(0.13)	(0.10)	(0.06)	(0.06)	(0.11)	(0.02)
				Mean osmotic potential after rehydration (MPa)					
Mighty Mo	-	-1.69	-	-1.66	-	-1.56	-	-1.93	-
		(0.04)		(0.02)		(0.03)		(0.01)	
Ohio Red	-	-1.62	-	-1.90	-	-1.49	-	-1.71	-
		(0.07)		(0.18)		(0.02)		(0.03)	
Platte	-	-1.63	-	-1.64	-	-1.38	-	-1.78	-
		(0.02)		(0.26)		(0.03)		(0.02)	
Rock Island	-	-1.69	-	-1.70	-	-1.47	-	-1.86	-
		(0.01)		(0.05)		(0.05)		(0.07)	
Tippecanoe	-	-1.74	-	-1.79	-	-1.78	-	-1.83	-
		(0.06)		(0.13)		(0.07)		(0.05)	
				Mean dry weight fraction					
Mighty Mo	0.267	-	0.285	0.336	0.260	0.276	0.269	0.288	0.336
	(0.004)		(0.011)	(0.034)	(0.009)	(0.004)	(0.006)	(0.004)	(0.019)
Ohio Red	0.256	-	0.281	0.322	0.254	0.277	0.277	0.307	0.324
	(0.002)		(0.010)	(0.011)	(0.007)	(0.001)	(0.004)	(0.003)	(0.003)
Platte	0.264	-	0.268	0.306	0.245	0.267	0.269	0.290	0.313
	(0.003)		(0.011)	(0.006)	(0.005)	(0.008)	(0.002)	(0.001)	(0.003)
Rock Island	0.259	-	0.293	0.335	0.244	0.285	0.281	0.301	0.331
	(0.013)		(0.006)	(0.026)	(0.009)	(0.003)	(0.001)	(0.002)	(0.001)
Tippecanoe	0.260	-	0.306	0.288	0.276	0.280	0.286	0.291	0.318
	(0.005)		(0.006)	(0.028)	(0.013)	(0.010)	(0.004)	(0.004)	(0.005)

Table A.7. Mean leaf water potential, osmotic potential (MPa), and dry weight fraction during 1989 growing season. Standard errors of mean are shown in parentheses (number of leaves = 7).

Clone	May 30	Jun 13	Jun 27	Jul 11	Jul 28	Aug 9	Aug 21
			Mean predawn water potential (MPa)				
Ohio Red	-0.38	-0.06	-0.03	-0.43	-0.44	-0.66	-0.48
	(0.02)	(0.01)	(0.00)	(0.04)	(0.03)	(0.06)	(0.06)
Platte	-0.26	-0.04	-0.01	-0.58	-0.51	-0.68	-0.59
	(0.02)	(0.01)	(0.01)	(0.03)	(0.02)	(0.05)	(0.03)
Tippecanoe	-0.35	-0.04	-0.02	-0.57	-0.54	-0.69	-0.64
	(0.01)	(0.01)	(0.00)	(0.04)	(0.04)	(0.10)	(0.06)
			Mean predawn osmotic potential (MPa)				
Ohio Red	-	-1.64	-1.37	-1.64	-1.65	-2.10	-1.98
		(0.02)	(0.05)	(0.03)	(0.04)	(0.02)	(0.02)
Platte	-	-1.75	-1.53	-1.77	-1.78	-2.10	-2.04
		(0.03)	(0.03)	(0.03)	(0.03)	(0.05)	(0.03)
Tippecanoe	-	-1.76	-1.63	-1.83	-1.77	-2.08	-2.08
		(0.02)	(0.01)	(0.03)	(0.02)	(0.02)	(0.03)
			Mean osmotic potential after rehydration (MPa)				
Ohio Red	-	-1.68	-1.44	-1.50	-1.65	-	-1.94
		(0.05)	(0.05)	(0.02)	(0.02)		(0.02)
Platte	-	-1.73	-1.52	-1.63	-1.71	-	-1.90
		(0.04)	(0.03)	(0.03)	(0.04)		(0.02)
Tippecanoe	-	-1.85	-1.63	-1.68	-1.75	-	-1.92
		(0.02)	(0.02)	(0.03)	(0.03)		(0.05)
			Mean dry weight fraction				
Ohio Red	0.274	0.308	0.308	0.269	0.302	0.319	0.327
	(0.006)	(0.003)	(0.006)	(0.003)	(0.005)	(0.004)	(0.002)
Platte	0.287	0.308	0.303	0.278	0.311	0.318	0.315
	(0.003)	(0.002)	(0.004)	(0.007)	(0.003)	(0.003)	(0.006)
Tippecanoe	0.262	0.302	0.295	0.279	0.301	0.319	0.318
	(0.003)	(0.003)	(0.001)	(0.004)	(0.005)	(0.007)	(0.004)

Table A.8. Mean height (cm) during 1988 growing season. Standard errors of mean are shown in parentheses (number of plants = 9).

	Mean height (cm) 1988				
Clone	May 7	Jun 7	Jul 11	Aug 10	Sep 19
Mighty Mo	25.42	45.33	63.47	98.21	133.21
	(4.59)	(3.59)	(4.87)	(6.50)	(10.99)
Ohio Red	12.38	27.33	37.11	69.85	98.92
	(2.76)	(2.13)	(2.20)	(3.20)	(6.61)
Platte	35.22	52.00	66.82	108.37	146.61
	(1.57)	(2.92)	(3.47)	(8.36)	(13.53)
Rock Island	24.28	37.22	54.68	101.32	139.42
	(0.97)	(2.29)	(4.33)	(8.01)	(8.45)
Tippecanoe	30.78	46.89	60.33	97.23	128.55
	(1.08)	(1.50)	(3.38)	(5.57)	(8.51)

Table A.9. Mean height (cm) during 1989 growing season for the same plants whose height was measured in 1988. Standard errors of mean are shown in parentheses (Number of plants = 9).

	Mean height (cm) 1989			
Clone	May 11	June 11	July 12	August 9
Mighty Mo	153.11	187.82	271.22	307.40
	(11.42)	(15.78)	(20.95)	(24.05)
Ohio Red	120.23	153.11	245.25	280.56
	(7.56)	(11.43)	(17.93)	(22.49)
Platte	170.60	209.27	283.07	311.49
	(14.99)	(19.12)	(22.93)	(23.73)
Rock Island	158.19	181.33	253.72	276.22
	(9.19)	(11.03)	(14.93)	(12.38)
Tippecanoe	148.87	180.48	260.49	291.22
	(9.49)	(11.60)	(17.61)	(20.25)

Table A.10. Mean water potential, osmotic potential, and dry weight fraction at solar noon (1 P.M. Central Daylight Time) during 1988 growing season. Standard errors of mean are shown in parentheses (number of leaves = 3).

Clone	June 14	June 28
	Mean water potential (MPa)	
Mighty Mo	-1.24 (0.03)	-1.29 (0.08)
Ohio Red	-1.25 (0.03)	-1.28 (0.05)
Platte	-1.25 (0.02)	-1.18 (0.10)
Rock Island	-1.41 (0.02)	-1.61 (0.03)
Tippecanoe	-1.35 (0.05)	-1.48 (0.04)
	Mean osmotic potential (MPa)	
Mighty Mo	-2.05 (0.03)	-2.28 (0.10)
Ohio Red	-2.09 (0.08)	-2.18 (0.02)
Platte	-2.07 (0.06)	-2.21 (0.04)
Rock Island	-2.06 (0.04)	-2.30 (0.05)
Tippecanoe	-2.32 (0.05)	-2.35 (0.03)
	Mean dry weight fraction	
Mighty Mo	0.258 (0.006)	0.284 (0.007)
Ohio Red	0.245 (0.010)	0.291 (0.011)
Platte	0.252 (0.003)	0.272 (0.002)
Rock Island	0.261 (0.010)	0.293 (0.016)
Tippecanoe	0.251 (0.003)	0.293 (0.005)

APPENDIX B: CALCULATION OF CONFIDENCE LIMITS FOR PREDICTED INJURY INDEX VALUES AT SELECTED RELATIVE WATER CONTENTS

The following is an example of a procedure that was used to calculate confidence limits for a true mean value of injury index at a selected value of relative water content (RWC), partially based on a SAS (SAS Institute Inc. 1985)[1] output.

Predictive equations based on injury index (dependent variable) and RWC (independent variable) were selected from a stepwise regression. Only those variables with significant ($\alpha \leq 0.05$) contribution were selected. After the equations were selected, a print-out of the generalized inverse of X'X matrix was obtained using the inverse option of the general linear models procedure for each clone at each sampling date (this example is for the Tippecanoe clone August 21, 1989 sample).

The independent variables of interest (in this example 55, 3025, and 166375 for a RWC of 55%; and 60, 3600, and 216000 for 60% RWC since the equation was cubic) were input in a matrix form, transposed and multiplied by the generalized inverse of the X'X matrix. The value obtained was multiplied by the mean square of error for the clone.

The output was:

Inverse matrix

	COL1	COL2	COL3	COL4
ROW1	-262.5	-11.6508	0.1662	-7.6E-04
ROW2	-11.6508	0.5199	-0.007452	3.4E-05
ROW3	0.1662	-0.007452	1.1E-04	-5.0E-07
ROW4	-7.6E-04	3.4E-05	-5.0E-07	2.3E-09

Variance at RWC 55% = 1.0089

[1]SAS User's Guide: Statistics, Version 5 ed. 1985. SAS Institute Inc., Cary, NC.

Variance at RWC 60% = 1.0980

Predicted injury indexes were 18.64% and 11.21% at RWC 55% and 60%, respectively. The t-value from a t-distribution table for degrees of freedom of 31 and one-sided probability (α = 0.025) was 2.04.

Using this information, confidence limits were calculated from:

$18.64 \pm 2.04 * 1.0089^{0.5}$ (for 55% RWC)

$11.21 \pm 2.04 * 1.0980^{0.5}$ (for 60% RWC)

The confidence limits are 16.59 to 20.69%, and 9.07 to 13.35% for 55% and 60% RWC, respectively.